# $^1$H NMR and X-ray conformational analyses of (+)-corydalic acid methyl ester, a 6,7-secoberbine alkaloid

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**Conformational analysis of (+)-corydalic acid methyl ester (1) by $^1$H NMR data indicated that 1 in chloroform solution at room temperature exists in a conformational equilibrium. The NOEs in the NOESY spectra of 1 and the temperature dependence of the NMR spectral pattern suggested that rotation of the ring A moiety around the C(14)–C(15) bond is obstructed by two neighbouring methyl groups on the N(7) and C(13) positions. The structure of 1 was determined to be methyl (6*R*-*trans*)-6-(6,7,8,9-tetrahydro-6,8-dimethyl-1,3-dioxolo[4,5-*h*]isoquinolin-7-yl)-1,3-benzodioxole-5-acetate by X-ray crystal structure analysis. The crystal conformer of 1 agrees well with one of the two stable conformers derived from NMR analysis and empirical energy calculations. The function of 6,7-secoberbine type alkaloids for the biosynthetic pathway from protoberberine type into the hexahydrobenzo[*c*]phenanthridine type is discussed in relation to their conformational features.**

The corydalic acid methyl ester **1**, isolated from *Corydalis incisa* (*papaveraceae*),[1] is one of the 6,7-secoberbine-type alkaloids.[2] The 6,7-secoberbine-type results from the fission of the C(6)–N(7) bond in the skeleton of the protoberberine-type (Fig. 1). It was believed that **1** is derived from a hypothetical intermediate **2** in the biosynthetic conversion route from tetrahydrocorysamine **3** to corynoline **4**.[3] Despite interest in its biosynthesis, however, the stereostructure of **1** remains uncertain. Thus, the elucidation of its detailed molecular conformation would provide biosynthetic information on the recyclization process to the hexahydrobenzo[*c*]phenanthridine-type alkaloid **4**.

The present paper deals with the molecular conformation of (+)-corydalic acid methyl ester **1**, using $^1$H NMR spectroscopic, X-ray crystal analysis and conformational energy calculation methods. We also discuss the biosynthetic route from 6,7-secoberbine-type intermediate **2** to **4**, based on the conformational analysis of **1**.

## Results and discussion

### $^1$H NMR studies

The geometrical structures of 6,7-secoberbine-type alkaloids have previously been elucidated by chemical correlation and total syntheses.[1,4–10] However, conformational questions remain. As for the stereostructure of **1**, (*i*) the ring conformation of ring B, (*ii*) the orientation of substituents of ring B, and (*iii*) the rotation around the bond C(14)–C(15) must be defined to provide the exact conformation.

The NMR spectra of **1** in $CDCl_3$ were reinvestigated to gain detailed information on the solution conformation. The chemical shifts and coupling constants of **1** are summarized in Table 1. The signal assignments were based on COSY and NOESY data.

The vicinal coupling constant between the protons C(13)H and C(14)H is $J = 8.0$ Hz which revealed a di-axial relationship.[11] The di-axial value down to 8 Hz may be attributed to the presence of the adjacent nitrogen and aryl substituents. In addition, the NOESY data can be used to clearly distinguish between the pseudo-axial-proton on ring B and the pseudo-equatorial-one. The partial NOESY spectra of **1** are shown in Fig. 2.

**Table 1** $^1$H NMR data for **1** in $CDCl_3$ at 23 °C

| Proton | $\delta_H$ (Multiplicity, *J*/Hz)[a] |
|---|---|
| C(21)$H_3$ | 1.048 (d, $J_{21–13}$ 7.0) |
| N–C(23)$H_3$ | 2.084 (s) |
| C(13)H | 3.050 (dq, $J_{13–14}$ 8.0, $J_{13–21}$ 7.0) |
| C(14)H | 3.205 (d, $J_{14–13}$ 8.0) |
| C(8)Hb | 3.394 (d, $J_{8b–8a}$ 15.5) |
| CO–OC(22)$H_3$ | 3.662 (s) |
| C(5)Hb | 3.680 (ABq, $J_{5b–5a}$ 16.0) |
| C(5)Ha | 3.775 (ABq, $J_{5a–5b}$ 16.0) |
| C(8)Ha | 4.040 (d, $J_{8a–8b}$ 15.5) |
| O–C(19)$H_2$–O | 5.962 (s) |
| O–C(20)$H_2$–O | 5.956 (dd, *J* 13.0, 1.5) |
| C(11)H | 6.708 (ABq, $J_{11–12}$ 8.0) |
| C(12)H | 6.741 (ABq, $J_{12–11}$ 8.0) |
| C(4)H | 6.738 (s) |
| C(1)H | 6.906 (br s) |

[a] Multiplicity abbreviations: br = broad; d = doublet; s = singlet; q = quartet.

NOEs were clearly observed for the C(8)Hb–C(14)H (*a*) and C(12)H–C(21)$H_3$ (*b*) proton pairs, suggesting their close proximity, namely 1,3-diaxial correlation between C(8)Hb and C(14)H, and pseudo-equatorial orientation of C(21)$H_3$ parallel to the C(12)H on the aromatic ring C. The NMR data suggest the half-chair conformation for the ring B and pseudo-axial orientation for the three protons of C(8)Hb, C(13)H and C(14)H.

Concerning the molecular conformation of **1**, the spatial arrangement between the rings A and B/C is of special interest. Since the single bond C(14)–C(15) connects the rings A and B/C in the molecule, it could in principle be freely rotating. In practice, however, free rotation is inhibited by steric hindrance between the neighbouring substituted groups.

NOEs were also observed between C(14)H and both of the C(5)$H_2$ (*c* and *c′*) protons and between C(1)H and C(13)H (*d*) proton pairs. This spatial arrangement suggests a rotamer (Type 1) that has a torsion angle C(16)–C(15)–C(14)–C(14)H of *ca.* 0° (Scheme 1).

On the other hand, the NOE (*e*) [C(1)H and C(14)H pair], shown clearly in Fig. 2, suggests the existence of another

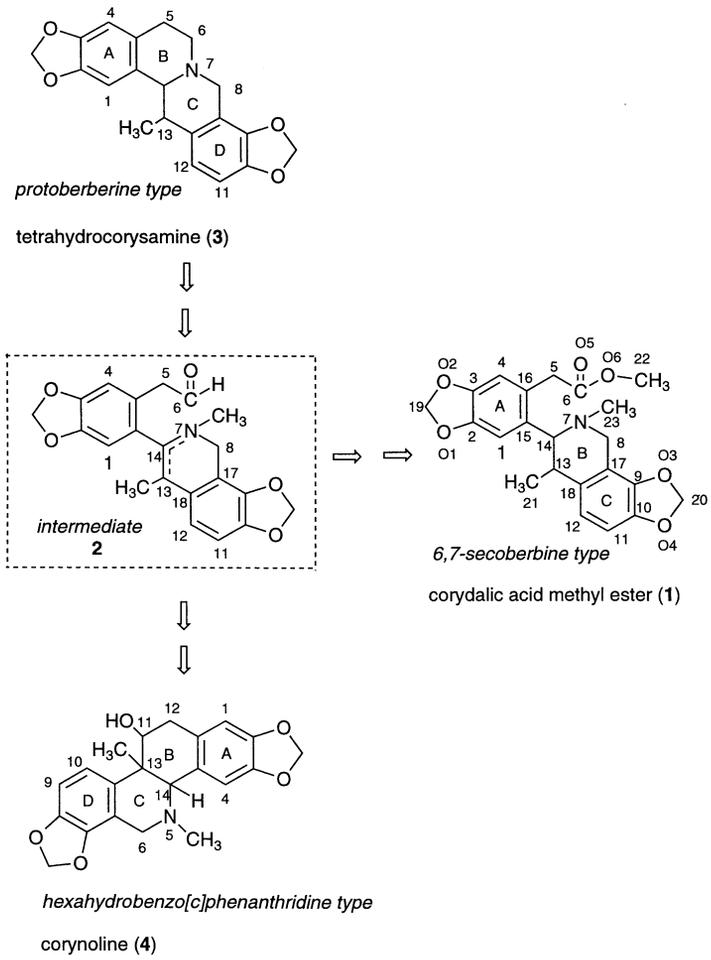



**Fig. 1** Biogenetic relationship between protoberberine-, hexahydrobenzo[*c*]phenanthridine- and 6,7-secoberbine type alkaloids. Atomic numbering is also given.

rotamer around the C(14)–C(15) bond, *i.e.* a rotamer (Type 2) with a torsion angle C(16)–C(15)–C(14)–C(14)H of *ca.* 180°. The population of *ca.* 7:3 (Type 1:Type 2) could be estimated at 23 °C from the respective NOE intensities, provided that the C(1)H–C(14)H distance is nearly equal to the C(1)H–C(13)H distance.

The peaks C(1)H, C(14)H, C(5)Ha and C(5)Hb in $CDCl_3$ appear as broad signals at room temperature, and at 55 °C these are sharpened and shifted to lower [C(5)Hb = 0.002, C(14)H = 0.006 ppm] or higher field [C(5)Ha = 0.002, C(1) = 0.007 ppm; at 0 °C, the signal from C(1)–H shows the broadest peak as a coalescence point (Fig. 3)]; unfortunately, we could not measure NMR spectra at below 0 °C because of the limitations of the NMR instrument, although experiments at below the coalescence point might be expected to provide useful information on the two rotamers.

These NMR data show that two species of **1** are in conformational equilibrium state in $CDCl_3$, where they adopt the same conformation for ring B and a different rotation angle of ring A with respect to the ring B/C, *i.e.* a major Type 1 conformer (evaluated by the NOE peak height in NOESY) having C(16)–C(15)–C(14)–C(14)H = *ca.* 0° and a minor Type 2 one having C(16)–C(15)–C(14)–C(14)H = *ca.* 180°.

## X-Ray analysis

In order to determine the stereostructure of the 6,7-secoberbine-type alkaloid, (+)-corydalic acid methyl ester **1** was subjected to X-ray crystallographic analysis.

Fig. 4 shows a stereoscopic view of the molecule of **1**. The geometry of **1** was shown to be methyl (6*R*-*trans*)-6-(6,7,8,9-tetrahydro-6,8-dimethyl-1,3-dioxolo[4,5-*h*]isoquinolin-7-yl)-1,3-benzodioxole-5-acetate, which is consistent with that elucidated by chemical correlation and synthetic methods.[1,4–10] The absolute configuration at C(13) and C(14) of **1** is set to the same configuration revealed[12] from a derivative from **1**. The ring B of **1** adopts a half-chair conformation as shown in Fig. 5.

The three substituents of the ring B, N(7)–methyl, C(13)–methyl and C(14)–phenyl (that is ring A), are all in the pseudo-equatorial orientation.

The torsion angles around the bond C(14)–C(15) and the dihedral angle between rings A and C provide us with information concerning the rotational isomer of **1**. The angles, N(7)–C(14)–C(15)–C(1) = 52.6(2)°, N(7)–C(14)–C(15)–C(16) = −132.7(3)°,C(13)–C(14)–C(15)–C(1) = −68.8(3)°,C(13)–C(14)–C(15)–C(16) = 106.0(3)°, show that the ring A plane is almost perpendicular to the plane of rings B/C and the methoxycarbonyl methyl group on C(16) in ring A is located at the β side of the rings B/C. The dihedral angle between the least-squares mean planes defined by ring A and C is 90.8(2)°. This angle also shows the perpendicular relationship between rings A and C. This crystal structure corresponds to the Type 1 conformer derived from the NMR analysis.

In the crystal structure, the C(5)–C(6)O(5)–$OCH_3$ group is located at the β side, and electrostatic interaction between the lone pair of N(7) on the α side and the C(6)=O(5) carbonyl group is not observed [N(7)–C(6) = 4.190, N(7)–O(5) = 4.504 Å].

## Conformational energy calculation

Stable conformers of **1** in the solution and in the solid state were investigated by NMR and X-ray analyses, and to further define the stable rotamer around the bond C(14)–C(15) of **1**,

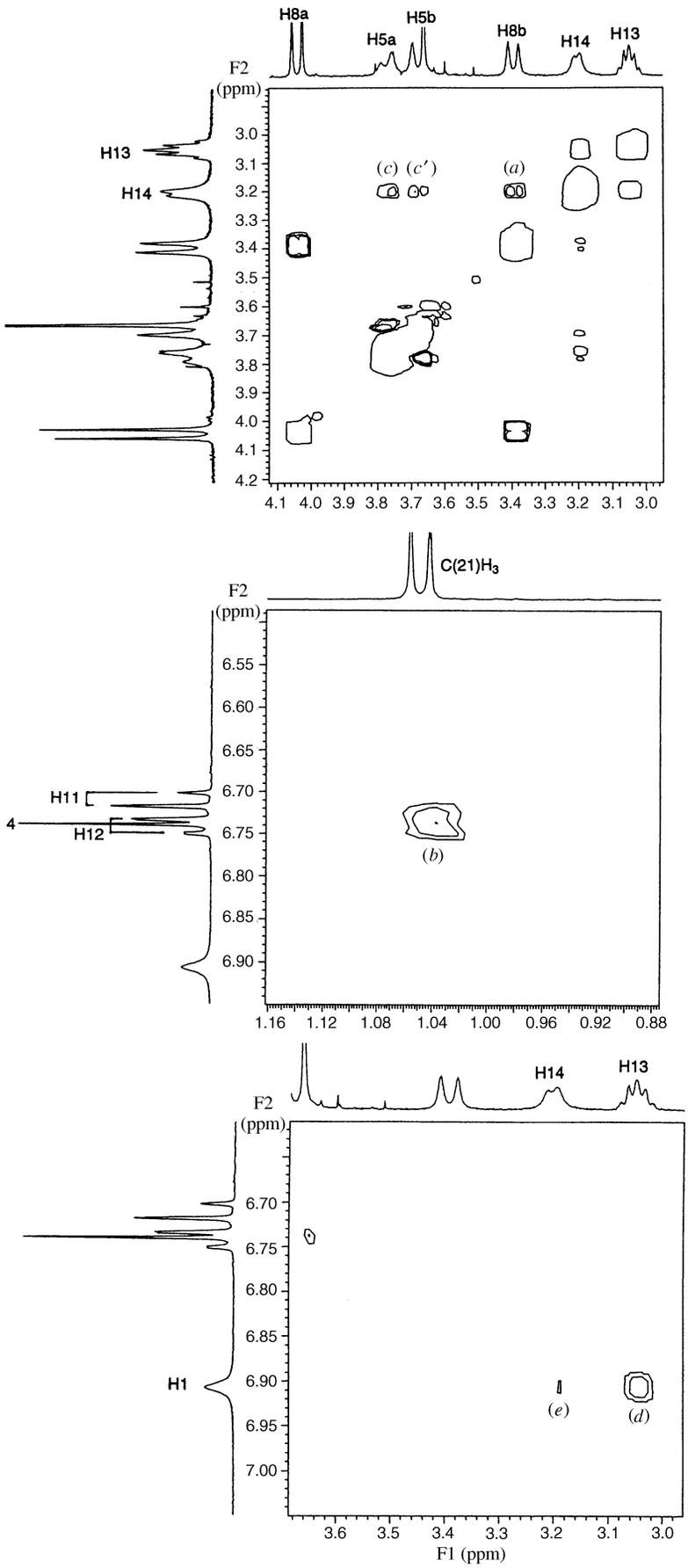
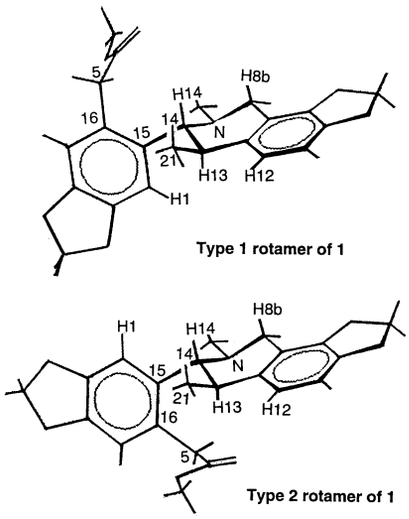



**Fig. 2** Partial NOESY spectra of **1** at 23 °C. (*a*) C(8)–Hb and C(14)–H, (*b*) C(12)–H and C(13)–$CH_3$, (*c*) and (*c*′) C(5)–$H_2$ and (14)–H, (*d*) C(1)–H and C(13)–H, (*e*) C(1)–H and C(13)–H.

the variation in total energy accompanying the rotation around the bond was examined. As a model compound for the calculation, the compound **5** (Fig. 6), which substitutes a methyl group for the methoxycarbonyl methyl group, was selected,




**Scheme 1**

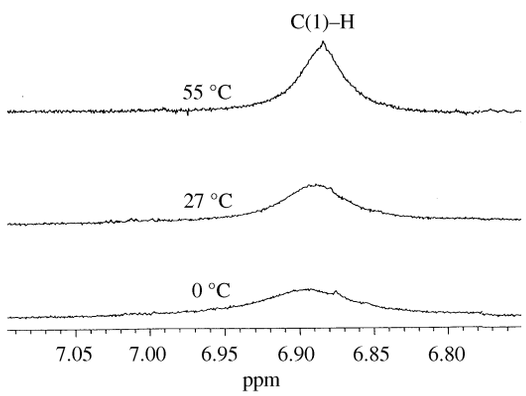



**Fig. 3** C(1)–H NMR spectra of **1** at 0, 27 and 55 °C

based on the lack of a specific interaction between the methoxycarbonyl methyl group and N(7) in the crystal. The energetically stable rotamers of **5** and of its *N*-protonated form were calculated by CNDO/2 method, as a function of the torsion angle ($\omega$) C(13)–C(14)–C(15)–C(16) rotated in increments of 30° from 0 to 360°.

The variations in the total energy of both **5** and its *N*-protonated form are shown in Fig. 6. The energy profile of **5** shows a continuous function having the two most stable regions B1 and B2 ($\omega$ = *ca.* 90–120° and *ca.* 270–300°) and the two unstable regions P1 and P2 ($\omega$ = *ca.* 60° and *ca.* 210°). This result suggests that **5** adopts an equilibrium state for the rotamers in the B1 and B2 regions. Although the semiempirical CNDO/2 energy calculations may not show the energy barrier accompanied by the rotation of $\omega$ torsion angles, realistically, it could indicate that **5** has two stable conformations between both B regions, the energies of which are nearly identical, and the interconversion between these two rotamers (B1 and B2) is actually impossible on a change in environment such as a change in solvent.

The profile of the *N*-protonated form of **5** is virtually the same as that of **5**. It suggests that the pH in solvent should have no significant influence on the conformational equilibrium in **5**.

The present results for **5** further suggest that the same energy pattern is also applicable to the energy pattern of **1**, as judged from the NMR experiments of **1**, and support **1** adopting the conformational equilibrium of B1 type rotamer (Type 1) and B2 type rotamer (Type 2) in the solution.

From the X-ray crystal analysis of **1**, the torsion angle C(13)–C(14)–C(15)–C(16) was shown to be 106.0(3)°. This value coincides with the angle of stable B1 region obtained from the calculation for model compound **5**. In the crystal, only one conformation was obtained, and this would be due to the crystal packing effect among the neighbouring molecules.

Following the elucidation of the biosynthesis of isoquinoline alkaloids,[13] we have reported some data on the stereostructural problems from the substrate side.[12,14]

Provided that the conformation of compound **5** is kept for compound **2**, which is a key intermediate compound between tetrahydrocorysamine **3** and corynoline **4** (Fig. 1), it could be assumed that **2** takes a conformational equilibrium state (B1 rotamer : B2 rotamer = 1 : 1), and racemic (±)-corynoline (**4**) is produced from only one kind of enantiomeric substrate, not from two enantiomeric isomers, *i.e.* (+)-(11*S*,13*R*,14*R*)-corynoline **4**[15] is produced from the B1 type rotamer of (+)-**2** in β-phase cyclization and (−)-(11*R*,13*S*,14*S*)-corynoline **4** is from the B2 type rotamer of (+)-**2** in α-phase (Fig. 7).

Corynoline **4** is actually isolated from the plant (*Corydalis incisa Pers.*, *Papaveraceae*) as an enantiomeric mixture, (+)-**4** (57%) and (−)-**4** (43%),[15] this being rare in natural products. Though not definitive, the present results suggest that respective conformers in the conformational equilibrium state are closely related to the recyclization mechanism of the (+)- and (−)-enantiomeric alkaloid molecules.

## Experimental

### $^1$H NMR spectroscopy

$^1$H NMR spectroscopic measurements were carried out on a Varian VXR-500 NMR spectrometer at 0, 23, 27 and 55 °C, and at 0.03 M in $CDCl_3$ solution. Experiments below 0 °C were not performed, because of the limitation of the thermoregulator.

The deuterium resonance of the solvent $CDCl_3$ was used as the lock signal, internal reference $SiMe_4$. Signal assignments were performed by two-dimensional correlated spectroscopy (COSY), where the estimated standard deviations are 0.001 ppm for the chemical shift and *ca.* 0.5 Hz for the coupling constant. The nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum was recorded in the phase-sensitive mode at 23 °C. The NOESY spectrum was measured with a mixing time of 500 ms.

For **1** at 55 °C; $\delta_H$ 3.059 (13H), 3.211 (14H), 3.399 (8bH), 3.656 ($OCH_3$), 3.682 (5bH), 3.773 (5aH), 4.037 (8aH), 6.741 (11H), 6.727 (12H), 6.734 (4H), 6.899 (1H).

### X-Ray analysis of 1

**Sample.** (+)-Corydalic acid methyl ester **1** was isolated from *Corydalis incisa* (*Papaveraceae*) according to the literature.[1] **1**; mp *ca.* 140.0–141.0 °C (acetone/light petroleum) colourless prisms.

The single crystals for X-ray studies were crystallized as transparent prisms from the mixed solvent methanol–chloroform–ethyl acetate at room temp. A single crystal with the dimension of 0.3 × 0.3 × 0.4 mm$^3$ was obtained.

**Crystal-structure determination and refinement.** The crystal data for (+)-corydalic acid methyl ester **1** are presented in Table 2. The crystal density was measured by the flotation method, using an aqueous KI solution. The cell dimensions and orientation matrix were calculated by the least-squares method from the angular values of 25 reflections collected with an AFC-5 diffractometer (Rigaku Co. Ltd.) using the graphite-monochromated Cu-Kα radiation. The intensities were collected in the $\omega$–2$\theta$ scan mode. The intensities of four standard reflections, measured at every 100 reflection intervals, remained constant to within ±1% of their mean values. The measured intensities within 2$\theta$ = 130° were then subjected to Lorentz and polarization corrections; no absorption correction was applied. The structure was solved by direct methods using the MULTAN program,[16] and refined by the full-matrix least-squares method with isotropic thermal parameters, and then by the

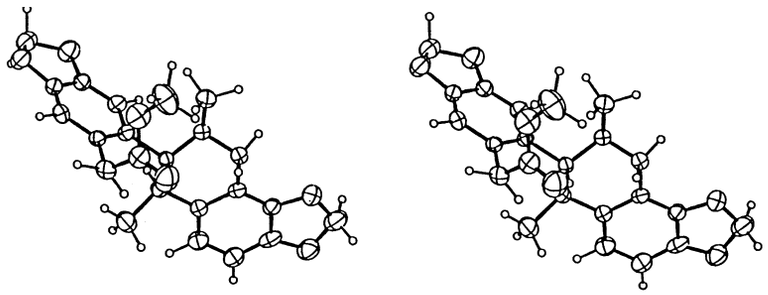


**Fig. 4** Stereoscopic molecular structure of (+)-corydalic acid methyl ester (**1**)

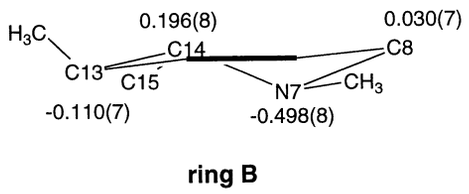



**Fig. 5** Schematic projection of ring *B* and deviation of each atom from the least-squares plane of the aromatic ring. The thick bar stands for the aromatic ring *C*. Deviations in Å, with esd values in parentheses.

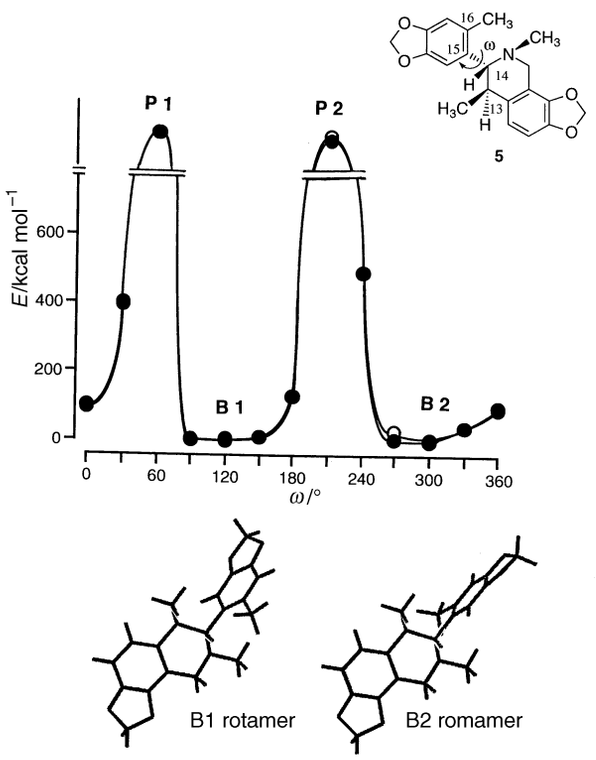



**Fig. 6** Calculated energy profile of **5** as a function of C(13)–C(14)–C(15)–C(16) ($\omega$). Each plot is depicted as the energy difference from the lowest energy of **5** (−153034.8 kcal mol$^{-1}$ for $\omega = 300°$) (filled circles) and *N*-protonated **5** (−153377.57 kcal mol$^{-1}$ for $\omega = 120°$) (open circles).

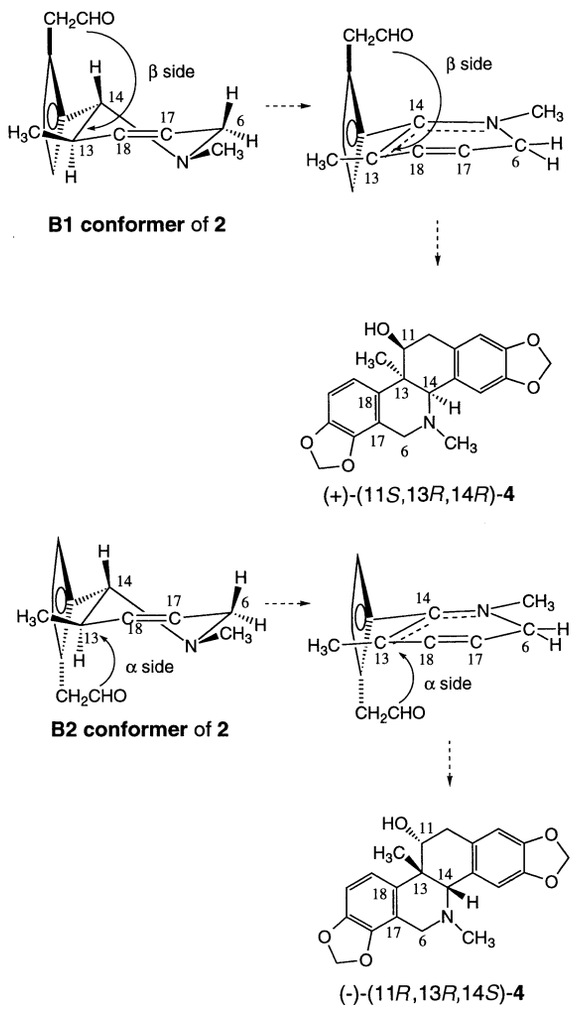



**Fig. 7** A proposal for biogenetic route from the two type conformers of (+)-**2** to (+)- and (−)-**4**

block-diagonal least-squares method with anisotropic ones using the SHELX-93 program.[17] The H-atom positions were located from the subsequent difference Fourier map. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$. None of the positional parameters shifted more than one-fifth of their standard deviation, and the maximum electron density in the final Fourier synthesis was 0.30 e Å$^{-3}$. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc., Perkin Trans. 2*, 1997, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 188/45. For all crystallographic computations, the UNICS programs[18] were used, and atomic scattering factors were from *International Tables for X-Ray Crystallography.*[19] All numerical calculations were carried out on an ACOS-3900 computer at the Computation Center of Osaka University and on a Micro VAX II computer at Osaka University of Pharmaceutical Sciences.

### Molecular orbital calculations

The total energies for various conformers of **5** were calculated by the CNDO/2 method,[20] as a function of torsion angle around the C(13)–C(14)–C(15)–C(16) ($\omega$) bond. The atomic coordinates of **5** and *N*-protonated **5** were constructed from the present X-ray result of **1** and used for the calculation. The stability of the electronic energy was used as a check for convergence in the iteration calculation. The total energies (kcal mol$^{-1}$) (1 cal = 4.184 J) of 13 different rotamers were computed

**Table 2** Crystal data of (+)-corydalic acid methyl ester (**1**)

| | |
|---|---|
| Formula | $C_{22}H_{23}O_6N$ |
| $M_r$ | 397.428 |
| Crystal system | Orthorhombic |
| Space group | $P2_12_12_1$ |
| $a$/Å | 15.251(3) |
| $b$/Å | 17.152(3) |
| $c$/Å | 7.618(1) |
| $V$/Å$^3$ | 1992.8(6) |
| $F$(000) | 840 |
| $\lambda$/Å | 1.5405 |
| $\mu$/cm$^{-1}$ | 7.61 |
| $Z$ | 4 |
| $D_c$/g cm$^{-3}$ | 1.325 |
| $D_m$/g cm$^{-3}$ | 1.320 |
| No. of obs. reflections | 1979 |
| No. of data with $F_o > 0.0$ | 1921 |
| No. of variables | 355 |
| $R$ | 0.0455 |
| $Rw$ | 0.0977 |

in increments of 30° of $\omega$ angle from 0°–360°, where the structure was not optimized at each of the torsional angles, because the rotatable bonds are limited to the methyl groups.

**5**; −152 939.79 kcal mol$^{-1}$ ($\omega = 0°$), −152 640.97 (30°), −152 037.26 (60°), −153 030.41 (90°), −153 034.08 (120°), −153 026.37 (150°), −152 903.26 (180°), −152 108.83 (210°), −152 547.81 (240°), −153 031.80 (270°), −153 034.79 (300°), −152 996.06 (330°), −152 939.79 (360°). *N*-Protonated **5**; −153 281.08 (0°), −152 978.53 (30°), −152 381.50 (60°), −153 373.91 (90°), −153 377.57 (120°), −153 370.30 (150°), −153 249.51 (180°), −152 435.74 (210°), −152 885.99 (240°), −153 352.93 (270°), −153 375.57 (300°), −153 338.49 (330°), −153 281.08 (360°).

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