6. Conformational Features of 7,8,13β,14α-Tetrahydro-N^7-methylcorysaminium, a Biosynthetic Intermediate to the Protopine-Type Alkaloid Corycavine

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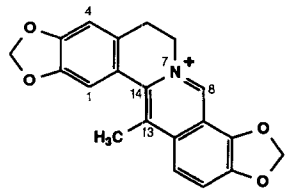
The crystal structure of (±)-7,8,13β,14α-tetrahydro-N^7-(^{13}C)methylcorysaminium iodide (^{13}C-**3a**·I) was investigated by X-ray analysis and thus the relative configuration (7*S**,13*S**,14*S**) established (*Fig. 1*). The conformation of **3a** was shown to have a *cis*-junction of the B/C rings and the rings A and D in an antiperiplanar position relative to the C(13)–C(14) bond ('*anti-cis*'), a twisted half-chair for ring B, and a half-chair for ring C (*Figs. 2* and *3*). Conformation analysis by ^{1}H-NMR data indicated that the crystal conformation of **3a** is also the preferred one in MeOH solution.

Introduction. – In the biosynthesis processes from protoberberine-type to protopine-type alkaloids, *N*-methylation of the protoberberine-type alkaloid is preceded by the hydroxylation at the C(14) position [1]. Interest in the *N*-methylated form of protoberberines as a biosynthetic intermediate has led to an investigation of the stereostructure, including the absolute configuration, of *N*-methylprotoberberinium.

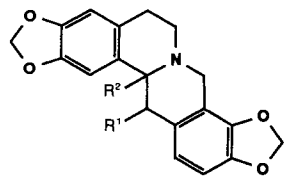
The tetrahydro derivative of corysamine (**1**), which is one of the protoberberine-type alkaloids isolated from *Coptis japonica* (*Ranunclaceae*) and *Corydalis incisa* (*Papaveraceae*) [2], obtained by chemical reduction, consists of four stereoisomers: (+)- and (−)-**2** [3] and (+)- and (−)-**3** [4]. This is due to the existence of two chiral C-atoms C(13) and C(14) in the structure[1]). In addition, the *N*-methyl product of this mixture of tetrahydro compounds consists of eight stereoisomers: (+)- and (−)-**2a** [5], **-2b**, **-3a** [4] [6], and **-3b**, due to the additional chiral N-atom N(7). It was shown that the enzyme which produces hydroxylation at C(14) allows a few of these isomers to be the precursors of the protopine-type alkaloid corycavinium (**4a**) [7]. In spite of the biosynthetic importance of compounds **2a**,**b** and **3a**,**b**, their configurations are still uncertain. The kind of steric features that affect their enzymatic reaction is not completely defined at present, but an understanding of the detailed molecular structure of the precursors of this process in the biosynthetic pathway would provide us with information on the stereospecificity of the enzyme reaction.

The present investigation was undertaken to determine, using X-ray crystal-structure analysis and ^{1}H-NMR data, the structure of 7,8,13β,14α-tetrahydro-N^7-methyl-

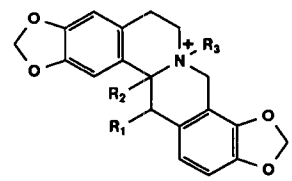
[1]) Arbitrary numbering; the systematic name of **1** is 6,7-dihydro-13-methylbis[1,3]benzodioxolo[5,6-*a*:4′,5′-*g*]-quinolizinium.



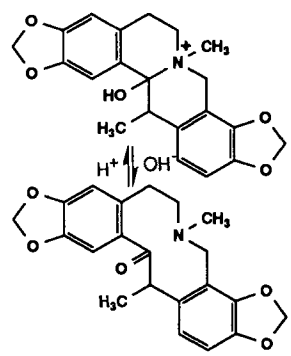
1, corysamine[1])



2 $R^1 = \beta$-Me_3, $R^2 = H$,
7,8,13α,14α-tetrahydrocorysamine
3 $R^1 = \alpha$-Me_3, $R^2 = \alpha$-H,
7,8,13β,14α-tetrahydrocorysamine



2a $R^1 = \beta$-Me, $R^2 = \alpha$-H, $R^3 = \beta$-Me,
cis-7,8,13α,14α-tetrahydro-$N^7\beta$-methylcorysaminium
b $R^1 = \beta$-Me, $R^2 = \alpha$-H, $R^3 = \alpha$-Me,
trans-7,8,13α,14α-tetrahydro-$N^7\alpha$-methylcorysaminium
3a $R^1 = \alpha$-Me, $R^2 = \alpha$-H, $R^3 = \alpha$-Me,
cis-7,8,13β,14α-tetrahydro-$N^7\alpha$-methylcorysaminium
b $R^1 = \alpha$-Me, $R^2 = \alpha$-H, $R^3 = \beta$-Me,
trans-7,8,13β,14α-tetrahydro-$N^7\beta$-methylcorysaminium



4a, corycavinium

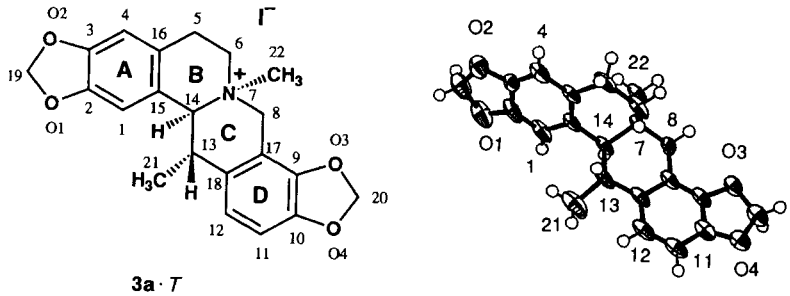
4b, corycavine

corysaminium (= 4,5,6,7,12bα,13β-hexahydro-5β,13α-dimethylbis[1,3]benzodioxolo-[5,6-*a*:4′,5′-*g*]quinolizinium; **3a**), one of the precursors of corycavinium (**4a**) [7].

Results and Discussion. – *X-Ray Analysis.* The geometrical structures of many protoberberine-type alkaloids were elucidated by chemical correlations and spectroscopic methods [8]. In the case of the *N*-methylated protoberberiniums, however, the structural questions still remain, since the usual spectral investigations (*e.g.* by IR, NMR, *etc.*) give little information about the B/C-ring-junction moiety, due to the structural characteristic of the angular *N*-methyl group on the B/C connection. To determine the configuration of *N*-methylated protoberberines, (±)-7,8,13β,14α-tetrahydro-N^7-(^{13}C)methylcorysaminium iodide (^{13}C-**3a**·I) was subjected to X-ray crystall-structure analysis.

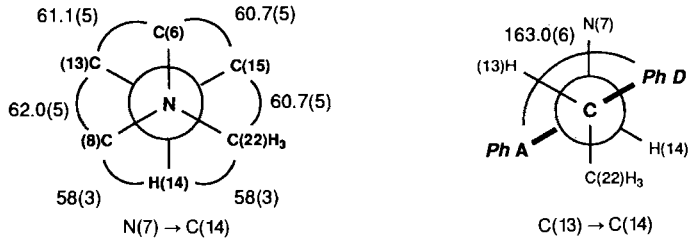
A perspective drawing of the structure of ^{13}C-**3a**·I with the numbering scheme is shown in *Fig. 1*. The geometry of the ion **3a** reveals the configuration (7*S**,13*S**,14*S**) with a *cis*-fused B/C quinolizidinium moiety and a *cis*-correlation of Me–C(13) and H–C(14). The structure of **3a** is consistent with that predicted by the NMR and CD data [6].

In contrast with the rigid conformation of a *trans*-fused B/C ring moiety in *N*-methylated protoberberinium ions, *cis*-fusion as in **3a** allows more flexibility. Thus, depending on the arrangement of the aromatic rings A and D, *i.e.*, antiperiplanar ('*anti*') or synclinal ('*syn*') to each other, two forms can exist (see '*anti-cis*' and '*syn-cis*' in *Fig. 2*). The torsion angles around the B/C ring junction axis N(7)–C(14) of **3a** are also shown in *Fig. 2*. The *cis*-fusion and the configurations at the quinolizidinium moiety of **3a** are consistent with



3a · *T*

Fig. 1. *Molecular structure of (±)-7,8,13β,14α-tetrahydro-* N^7*-methylcorysaminium iodide* (^{13}C-**3a**·I). Arbitrary numbering.



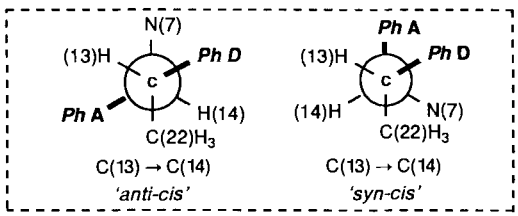


Fig. 2. *Conformation around the B/C ring junction of* **3a** *and* cis*-fused protoberberinium skeleton*

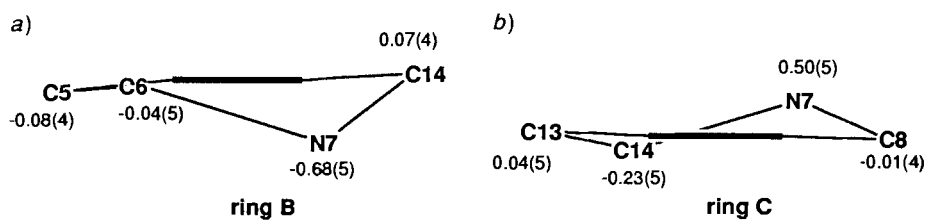


Fig. 3. *Schematic projection of rings B and C of* **3a** *and deviation of each atom from the least-squares plane of the aromatic ring.* The bold line stands for the aromatic ring, indicating in *a*) ring A and in *b*) ring D. Deviations in Å, with e.s.d.'s in parentheses.

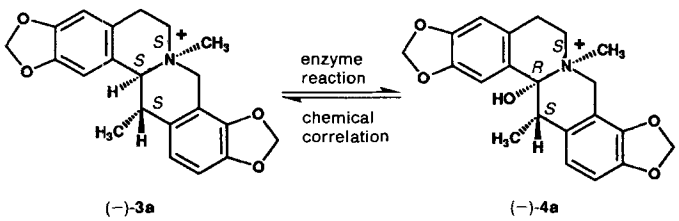
the '*anti-cis*' conformation, with a torsion angle $CH_3(22)$–N(7)–C(14)–H of 58(3)°. The dihedral angle between the mean planes of the aromatic rings A and D in the molecule is 74(1)°. Bond lengths and angles are similar to those of related compounds [9].

Ring B is in a half-chair and ring C in a twisted half-chair conformation (*Fig. 3*). Ring B is much more distorted than ring C. A similar '*anti-cis*' quinolizidinium conformation was also observed for **4a** [7].

Why the structure of **3a** takes an '*anti-cis*' conformation, but not a '*syn-cis*' one can be explained by considering *1*) the steric repulsion between the bonds H–C(1) and H–C(13) and between H_{ax}–C(5) and H_{ax}–C(8) and *2*) the energetically unfavorable axial orientations of Me–N(7) and Me–C(13) in the '*syn-cis*' conformation.

Since the relative configuration at N(7), C(13), and C(14) of the (±)-form **3a** was determined by the above X-ray analysis, the absolute configuration of the (–)-form (–)-**3** can be deduced to be (7*S*,13*S*,14*S*) by chemical correlation of (–)-**3a** with (–)-(7*S*,13*S*,14*R*)-corycavinium (**4a**) [7] (*Scheme*). This agrees with our previous CD spectral result [6].

Scheme. *Absolute Configuration of (–)-***3a**



In the crystal-packing diagram of ^{13}C-**3a**·I (*Fig. 4*), the shortest distance between anion and cation (4.3(5) Å) is found between N(7) and I^-. The layer consisting of the **3a** cations is arranged parallel to the *b*-axis, and I^- anions are inserted in the space among the layers by direct σ–π interaction. The coloration of this crystal is due to the $N^+ \cdots I^-$ ionic bonding in the structure.

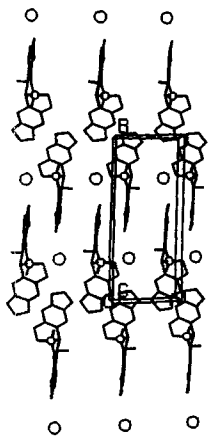


Fig. 4. *Crystal packing of* ^{13}*C-***3a**·*I viewed from* c*-axis.* Open circles represent I^- ions.

^{1}H-NMR Studies. Previous NMR spectroscopic analysis of the protoberberines and the *N*-methylated protoberberiniums dealt almost exclusively with explaining the chemical-shift and coupling-constant values [10]. This was not successful for the conformational analysis of *N*-methylated protoberberiniums because of the lack of practical information for NMR analysis. Too little is known concerning the structure possessing an angular *N*-methyl group on the B/C ring connection and an ionic bond N^+–I^- which influences the polarity of the compound. To overcome this deficiency, the NOESY spectrum of **3a** in MeOH was measured to determine the space arrangement of proton pairs in the structure.

The chemical shifts and coupling constants of **3a** are summarized in the *Exper. Part*, and the partial NOESY spectra are shown in *Fig. 5*. In the latter, two peaks were clearly observed for two proton pairs: H–C(1)/H–C(14) and Me–C(13)/H–C(12) (*Fig. 5a* and *5b*, resp.). The first one suggests that H–C(14) takes an equatorial orientation on ring B and is parallel to H–C(1). In addition, a similar parallel orientation between H–C(12) and equatorial Me–C(13) is deduced from the second NOE. These NOE's are consistent with the '*anti-cis*' conformation (see above) and with both rings B and C in half-chair form. The X-ray analysis of **3a** showed that these proton pairs are close together (2.40 Å for *H*–C(1) and H_{eq}–C(14) and 2.2–3.6 Å for *H*–C(12) and CH_3–C(13)). These observations show that the conformation of **3a** in solution seems to accord best with the X-ray-determined crystal conformation.

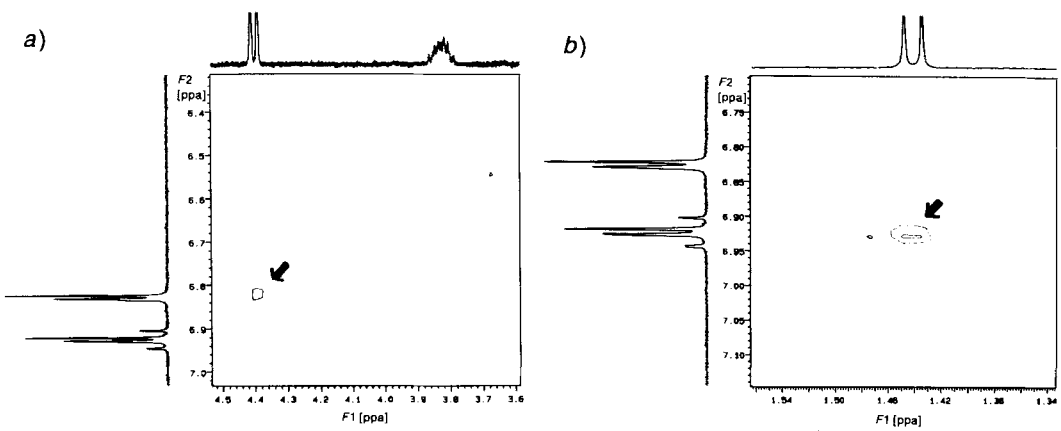


Fig. 5. *Partial NOESY spectra of* **3a** *· I in the region of two proton pais*: a) *H–C(1)/H–C(14) and* b) *H–C(12)/Me–C(13)*

Although several reports [5] described the isolation of *N*-methylated protoberberine-type alkaloids from plants, their biological significance is not fully recognized. The results obtained by the present study provide the following: Detailed comparison of the structure of **3a**, as a substrate, with that of **4a**, as a metabolite, showed that both structures agree very closely, not only concerning the chirality centers N(7), C(13), and C(14), but also regarding global molecular conformation. The OH addition to give **4a** proceeds with complete retention of the chirality and B/C ring conformation of **3a**.

To elucidate the biosynthetic route to isoquinoline alkaloids [7], we have provided data on the configurational problems on the substrate side.

Experimental Part

1. *X-Ray Analysis. Material.* The (±)-7,8,13β,14α-tetrahydro-N^7-(^{13}C)methylcorysaminium iodide (^{13}C-**3a**·I) was prepared according the [7], and the single crystals for X-ray studies were crystallized as brownish columns from MeOH at r.t.

Crystal-Structure Determination and Refinement. The crystal density was measured by the flotation method, using the solvent mixture benzene/bromobenzene/MeI. The crystal data for ^{13}C-**3a**·I is presented in the *Table*. The cell dimensions were refined by the least-squares method from the angular values of 25 reflections ($30 < 2\theta < 50$) collected with an *AFC-5* diffractometer (*Rigaku Co., Ltd.*). The intensities were collected with an ω-2θ scan mode. The intensities of 4 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. An empirical absorption correction using the program DIFABS [11] was applied. The

Table. *Crystal Data of (±)-7,8,13β,14α-Tetrahydro-*N^7*-(*^{13}*C)methylcorysaminium Iodide* (^{13}C-**3a**·I)

Formula	$^{12}C_{20}{}^{13}CH_{22}INO_4$	λ [Å]	1.5418
Mol. wt.	479.29	μ [mm^{-1}]	13.62
M.p. [°C]	315.0–317.2 (MeOH)	Z	2
Crystal system	triclinic	d_x [g·cm^{-3}]	1.694
Space group	$p\bar{1}$	d_m [g·cm^{-3}]	1.683(3)
Cell dimensions	$a = 7.571(1)$ Å	No. of obs. reflections	2971
	$b = 17.372(1)$ Å	No. of reflections with	
	$c = 7.467(1)$ Å	$F_o > 4\,\sigma(F_o)$	2825
	$\alpha = 96.76(1)°$	No. of variables	244
	$\beta = 105.30(1)°$	R	0.068
	$\gamma = 85.64(1)°$	wR	0.1736
V [Å^3]	939.6(2)	S	1.047
$F(000)$	480.0		

structure was solved by direct methods using the MULTAN 78 program [12], and refined by the full-matrix least-squares method with isotropic thermal parameters, and then by the block-diagonal least-squares method with anisotropic ones using the SHELX-93 program [13]. The H-atom positions were located from a 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 maximum electron density in the final *Fourier* synthesis was 0.48 e·Å^{-3}. Lists of final atomic coordinates of non-H atoms, anisotropic thermal parameters, bond lengths and angles, torsion angles, and structure factors were deposited as supplementary material. For all crystallographic computations, the UNICS program [14] were used, and atomic scattering factors were from 'International Tables for X-Ray Crystallography' [15]. All numerical calculations were carried out on an *ACOS-2020* computer at the Computation Center of Osaka University and on a *Micro VAX II* computer at Osaka University of Pharmaceutical Sciences.

2. *^{1}H-NMR Measurements. Varian-VXR-500* NMR spectrometer, at 23°, 0.001M soln. in CD_3OD·δ(D) of CD_3OD was used as the lock signal; δ (H) rel. to internal $SiMe_4$; signal assignments by 2D-correlated spectroscopy (COSY), estimated standard deviations for δ 0.001 ppm and for J *ca.* 0.5 Hz. Nuclear *Overhauser* enhancement and exchange spectroscopy (NOESY): in the phase-sensitive mode; mixing times 200 ms. ^{1}H-NMR: 6.931, 6.913 (*AB*('*q*'), $J = 8.0$, H–C(12), H–C(11)); 6.830 (*s*, H–C(4)); 6.824 (*s*, H–C(1)); 6.066 (*dd*, $J = 17.5, 1.0$, CH_2(20)); 6.022 (*s*, CH_2(19)); 4.408 (*d*, $J = 9.5$, H–C(14)); 3.245–3.860 (*AA'BB'* (*m*), CH_2(5), CH_2(6)); 3.306 (*d*, $J = 145.0$, $^{13}CH_3N$); 3.100 (*dq*, $J = 9.5, 6.9$, H–C(13)); 1.458 (*d*, $J = 6.9$, Me–C(13)).

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