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Gardneria Alkaloids. XII.[1] Carbon Magnetic Resonance Spectra of Gardneria Alkaloids. A Study on the Configuration of the Side Chain Double Bonds of Indole Alkaloids

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^{13}C-NMR spectra of several *Gardneria* alkaloids and their derivatives were studied. The chemical shifts of $C_{(15)}$ and $C_{(21)}$ were found to be diagnostic for the configuration of the side chain double bonds between $C_{(19)}$ and $C_{(20)}$. As a result of the present study the *E* configuration of the ethylidene side chain of chitosenine (V) was confirmed.

Keywords——CMR; indole alkaloid; Loganiaceae; *Gardneria nutans*; *Gardneria multiflora*; configuration of double bond; chitosenine; structure elucidation

Introduction

More than 18 alkaloids have been isolated from the plants of *Gardneria* spp. (Loganiaceae). The main base, gardneramine (I),[3,4] has been found in all kinds of *Gardneria* spp. so far examined[1,5] and the structure (I) was finally elucidated by X-ray crystal structure analysis of its cyanobromide.[3] One of the interesting features of the structure of gardneramine (I) is the *Z* configuration of its side chain double bond between $C_{(19)}$ and $C_{(20)}$, since it has long been believed that the ethylidene or hydroxyethylidene side chains of naturally occurring indole alkaloids generally possess *E* configuration.[6] Gardneramine (I) seems to be the first natural indole alkaloid with rigorously established *Z* side chain geometry. To date, six more *Gardneria* bases have been shown to have the same geometry by chemical correlation with gardneramine (I) in this laboratory.[1]

In addition to this group of alkaloids, several sarpagine type indole alkaloids have been found to coexist in the same genus.[5,7] The side chain geometry of gardnutine (X), a member of this group of alkaloids, was studied by means of the nuclear Overhauser effect (NOE) on the proton magnetic resonance (PMR) spectrum in this laboratory, and in this case "normal" *E* geometry was deduced.[7]

Obviously, to determine the geometry of the side chains by chemically correlating the compounds in question with compounds of known geometry is difficult task for minor bases which are present in the plants in small amounts, and so a more convenient method was

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[1] Part XI: S. Sakai, N. Aimi, K. Yamaguchi, K. Hori, and J. Haginiwa, *Yakugaku Zasshi*, **97**, 399 (1977).
[2] Location: *a*) *1-33, Yayoi-cho, Chiba*; *b*) *1-35-23, Nozawa, Setagaya-ku, Tokyo.*
[3] N. Aimi, S. Sakai, Y. Iitaka, and A. Itai, *Tetrahedron Lett.*, **1971**, 2061.
[4] *a*) S. Sakai, N. Aimi, A. Kubo, M. Kitagawa, M. Shiratori, and J. Haginiwa, *Tetrahedron Lett.*, **1971**, 2057; *b*) S. Sakai, N. Aimi, A. Kubo, M. Kitagawa, M. Hanasawa (née Shiratori), K. Katano, K. Yamaguchi, and J. Haginiwa, *Chem. Pharm. Bull.* (Tokyo), **23**, 2805 (1975).
[5] J. Haginiwa, S. Sakai, A. Kubo, K. Takahashi, and M. Taguchi, *Yakugaku Zasshi*, **90**, 219 (1970).
[6] A.A. Gorman, M. Hesse, and H. Schmid, "The Alkaloids-Vol. 1," Specialist Periodical Reports, The Chemical Society, London, 1971, p. 231.
[7] S. Sakai, A. Kubo, T. Hamamoto, M. Wakabayashi, K. Takahashi, H. Ohtani, and J. Haginiwa, *Chem. Pharm. Bull.* (Tokyo), **21**, 1783 (1973).

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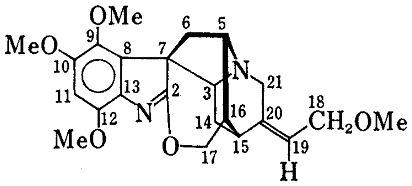
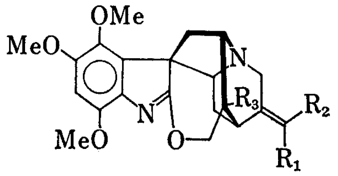
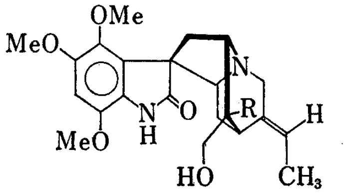
sought. We therefore studied the carbon magnetic resonance (CMR) spectra of *Gardneria* alkaloids and found them to be particularly useful for elucidation of the side chain geometry.



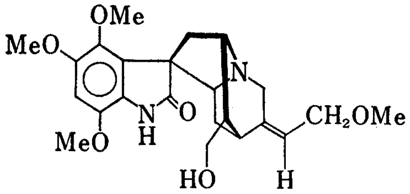
I gardneramine
(natural)



II : R_1=Me, R_2=R_3=H (natural)
III : R_1=H, R_2=Me, R_3=H
IV : R_1=Me, R_2=H, R_3=OH



V : R=OH chitosenine (natural)
VII : R=H



VI (natural)

Chart 1. Structures of Some Gardneria Alkaloids

Table I. ^{13}C Chemical Shifts of Gardneramine Type Substances[a]

Carbon	Compd.						
	I	II	III	IV	V[b]	VI	VII
2	178.7	178.8	178.8	178.2	180.5	180.1	180.0
3	62.4	63.0	62.6	62.0	66.0	66.4	66.3
5	60.4	59.9	60.6	70.0	69.0	58.3	58.6
6	31.2	30.3	31.7	30.8	33.5	32.1	32.0
7	63.0	63.0	63.0	62.0	59.2	58.3	58.3
8, 9, 10, 12, 13	133.1	133.2	133.2	132.4	131.0	122.5	122.4
	134.1	134.1	134.1	133.6	139.6	130.3	130.2
	139.4	139.6	139.4	139.3	141.0	139.0	138.9
	145.7	145.5	145.5	145.7	[d]	141.6	141.5
	150.5	150.5	150.5	150.6	[e]	150.1	150.0
11	100.2	100.2	100.2	99.8	99.6	98.4	98.2
14	32.2	32.2	32.2	28.2	24.1	23.7	23.4
15	36.9	29.8	36.8	37.4	34.0	34.0	26.5
16	42.0	41.8	42.2	[c]	75.8	43.5	42.8
17	72.0	72.3	72.1	74.9	65.2	61.6	61.7
18	68.0	12.7	12.3	12.3	12.6	68.1	12.5
19	115.0	112.1	112.5	118.1	113.0	113.4	110.2
20	147.6	142.0	143.2	136.6	142.6	148.9	142.7
21	46.4	49.4	46.5	48.9	50.3	46.8	49.4
18-OMe	58.2	—	—	—	—	57.8	—
Arom OMe	56.7	56.7	56.7	56.5	56.6	56.4	56.4
	57.6	57.6	57.6	57.3	57.0	57.0	56.9
	61.3	61.3	61.3	61.3	61.8	61.9	61.7

a) The δ values are in ppm downfield from TMS.
b) Measured in pyridine-d_5.
c, d, e) The corresponding signals are hidden in the peaks of the solvents.

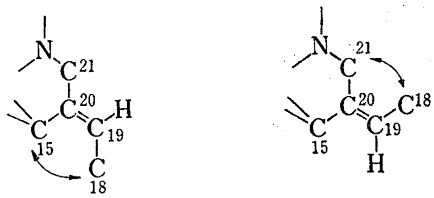
Results and Discussion

CMR Spectra of Gardneramine (I)[4] and Its Analogs. Elucidation of the Double Bond Geometry of Chitosenine (V)[8]

The chemical shifts of the carbon signals of seven compounds of this group are shown in Table I. The assignments have been made on the basis of chemical shift values and the multiplicities of the signals.

When the chemical shifts of $C_{(15)}$ signals of compounds I through III were compared, that of II[8,9] was observed to resonate at a higher field (29.8 ppm) than those of I (36.9 ppm) and III[9] (36.8 ppm). On the other hand, the chemical shift of the signal due to $C_{(21)}$ of II appeared at about 3 ppm lower field than those of I and III. A similar tendency was observed when the chemical shifts of the signals due to $C_{(15)}$ and $C_{(21)}$ of VI[4] were compared with those of the corresponding signals of the oxindole VII which was easily prepared from the natural iminoether II. Thus $C_{(15)}$ of VII resonated at a higher field (26.5 ppm) than the corresponding carbon of VI (34.0 ppm), while the signal due to $C_{(21)}$ of VII was observed at lower field (49.4 ppm) than that of the corresponding carbon of VI (46.8 ppm).

The above observation can be reasonably interpreted in terms of the well known γ effect on the allylic carbons ($C_{(15)}$ or $C_{(21)}$) caused by the substituent ($C_{(18)}$) on the double bond. In compounds in which the configuration of the double bond is *E*, that is, $C_{(18)}$ is oriented *cis* to $C_{(15)}$, the signal due to $C_{(15)}$ appears at higher field than that of the corresponding carbon in compounds with *Z* type double bond configuration. The reverse is true of the chemical shift of the $C_{(21)}$ signal.



E configuration **Z configuration**

Fig. 1. *E* and *Z* Configurations of the Side Chain Double Bonds in Gardneria Alkaloids

A few years ago, we deduced the structure (V) of an oxindole, chitosenine, which had been isolated from *Gardneria multiflora* (Japanese name: Chitosekazura).[8] This alkaloid also occurs in the plant as a monomeric component of a novel type of bisindole alkaloid, gardmultine.[10] However, the configuration of the ethylidene side chain remained unsettled in the structure of (V). To clarify this point, the CMR spectrum of the iminoether (IV),[8] which was derived from V *via* the mesylate, was measured and the data were compared with those of compounds I—III.

The signal due to $C_{(21)}$ of IV appeared at a position corresponding to $C_{(21)}$ situated *trans* to the side chain carbon ($C_{(18)}$). Thus *E* orientation of the side chain of chitosenine (V) was deduced.

Though in a less straightforward way, the same conclusion was derived from the chemical shift of $C_{(15)}$ of IV, which was observed at 37.4 ppm. This value might be taken as indicating *Z* configuration because of its close similarity to that of $C_{(15)}$ of I or III. Compound (IV), however, possesses a hydroxy group on $C_{(16)}$, β to $C_{(15)}$, which must cause a downfield shift of 5—10 ppm in the signal of $C_{(15)}$[11] and therefore the observed shift must be taken as the result of competition of the shielding γ effect by $C_{(18)}$ with the deshielding β effect due to the hydroxy group at $C_{(16)}$. This rationalization leads to the assignment of *E* geometry for the ethylidene side chain of IV.

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[8] S. Sakai, N. Aimi, K. Yamaguchi, H. Ohhira, K. Hori, and J. Haginiwa, *Tetrahedron Lett.*, **1975**, 715.

[9] S. Sakai, N. Aimi, K. Katano, H. Ohhira, and J. Haginiwa, *Yakugaku Zasshi*, 94, 225 (1974).

[10] S. Sakai, N. Aimi, K. Yamaguchi, E. Yamanaka, and J. Haginiwa, *Tetrahedron Lett.*, **1975**, 719.

[11] J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, N.Y., 1972, p. 139, 165.

Next, the CMR spectrum of chitosenine (V) itself was measured and the data were compared with those of the closely related oxindoles VI[4] and VII. In the spectrum of chitosenine iminoether (IV), the signal of the most characteristic quaternary carbon, $C_{(16)}$, was not observable due to overlapping with the peaks of the solvent (deuterio chloroform), but in the spectrum of V the corresponding carbon was clearly observed at 75.8 ppm as a singlet in the off-resonance spectrum. This and the other signals were quite consistent with the previously deduced structure (V)[8] of chitosenine. The signal due to $C_{(21)}$ of V was observed at 50.3 ppm, a position similar to that of $C_{(21)}$ of VII in which the double bond geometry was *E*, thus supporting *E* geometry of the side chain of V. As was the case with the iminoether derivative (IV), the shift value of $C_{(15)}$ of V was rather closer to that of the *Z* type compound (VI) than to that of the *E* isomer (VII). This apparent inconsistency can also be explained as a coincidental result of cancellation of the shielding γ effect due to $C_{(18)}$ by the deshielding β effect of the hydroxy group on $C_{(16)}$. Therefore an *E* configuration of chitosenine (V) was deduced.

Assignments of the other signals are shown in Table I. There are two methine signals, one at around 60 ppm and the other at around 63 ppm in the spectra of compounds I—III. In the spectrum of IV, the former was observed at 70.0 ppm while the latter remained practically unchanged. Based on this observation the peak which appeared at 60 ppm in the spectra of I—III can be ascribed to $C_{(5)}$, which is shifted downfield in IV by the deshielding β effect of the hydroxy group on $C_{(16)}$. This substituent can be considered to produce practically no effect on the chemical shift of $C_{(3)}$, to which the unchanged signal at 63 ppm can be assigned.

Differentiation of the methylene carbons of $C_{(6)}$ and $C_{(14)}$ in I—IV was tentatively done as follows. Comparison of the CMR data of II and IV showed that while the signal at 30.3 ppm of II remained unchanged in IV, the signal at 32.2 ppm of II was shifted to 28.2 ppm in the spectrum of IV. This observation can be explained in terms of the γ-antiperiplanar effect[12] of the $C_{(16)}$-hydroxy group. A molecular model revealed that the relative position between $C_{(14)}$ and the oxygen atom at $C_{(16)}$ in IV fits the requirement of this effect. Thus the signal at 32.2 ppm in II can be ascribed to $C_{(14)}$.

No attempt to differentiate the singlet aromatic carbons, $C_{(8)}$, $C_{(9)}$, $C_{(10)}$, $C_{(12)}$ and $C_{(13)}$, has been made. The three aromatic methoxyl carbons at $C_{(9)}$, $C_{(10)}$ and $C_{(12)}$ also remain unassigned.

Sarpagine Type Gardneria Alkaloids, VIII—XII

Gardneria nutans has been shown to contain three kinds of sarpagine type indole alkaloids, gardnerine (VIII), gardnutine (X) and hydroxygardnutine (XI), all of which possess an aromatic methoxyl group on $C_{(11)}$.[7] Recently, 18-hydroxygardnerine (IX), which was obtained as a $LiAlH_4$ reduction product of XI, was also found to exist in *G. nutans.*

Determination of the geometry of the side chain double bonds of this group of alkaloids has already been done by NOE experiment on the PMR spectrum. Thus irradiation at the signal due to $C_{(18)}$ methyl protons of gardnutine (X) caused an increase in the signal area of the $C_{(15)}$ proton by 13%, indicating E geometry of the side chain double bond.[7] The configurations of the double bonds of other members of this class of alkaloids have been determined by chemically correlating them with gardnutine (X). As a result, all of the natural *Gardneria* bases of this structural type have been shown to possess "normal" *E* geometry of the side chain double bonds.

The above conclusion was based on NOE experiments on the single compound (X), and so it was considered desirable to confirm the results by CMR spectroscopy. We were also

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[12] E.L. Eliel, W.F. Bailey, L.D. Kopp, R.L. Willer, D.M. Grant, R. Bertrand, K.A. Christensen, D.K. Dalling, M.W. Duch, E. Wenkert, F.M. Scell, and D.W. Cochran, *J. Am. Chem. Soc.*, **97**, 322 (1975).

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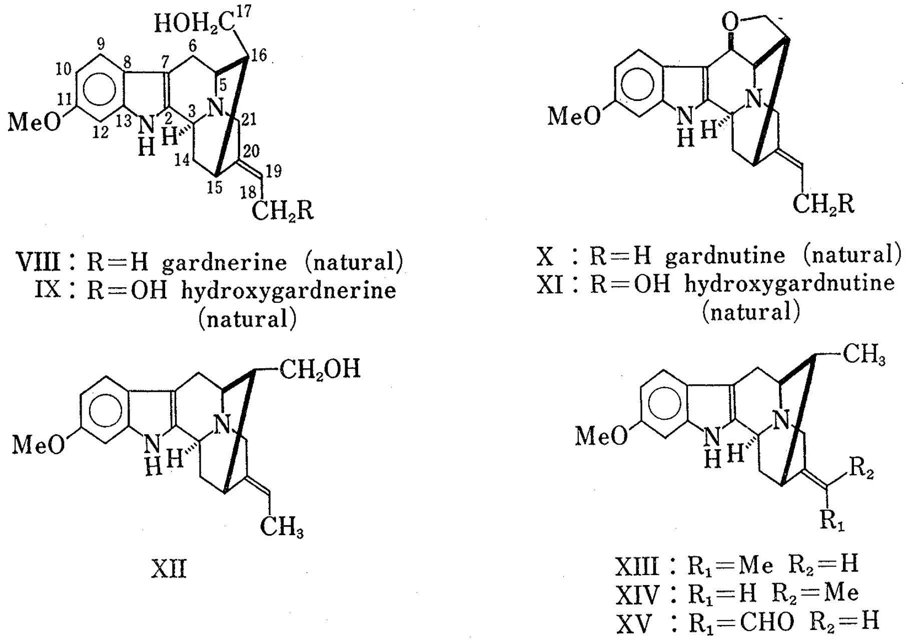
interested in measuring the CMR spectra of some abnormal reaction products obtained in the course of our chemical correlation studies.

The CMR spectrum of gardnerine (VIII) unambiguously confirmed *E* double bond geometry in terms of the chemical shift of $C_{(15)}$ and $C_{(21)}$. Hydroxygardnerine (IX) showed corresponding signals at similar positions, proving the same geometry of the double bond.



VIII : R=H gardnerine (natural)
IX : R=OH hydroxygardnerine (natural)

X : R=H gardnutine (natural)
XI : R=OH hydroxygardnutine (natural)

XII

XIII : R_1=Me R_2=H
XIV : R_1=H R_2=Me
XV : R_1=CHO R_2=H

Chart 2. Structures of Some Gardneria Alkaloids

TABLE II. ^{13}C Chemical Shifts of Gardnerine-type Substances$^{a)}$

Carbon	Compd.					
	VIII$^{b)}$	IX$^{b)}$	XI$^{c)}$	XII$^{b)}$	XIII	XIV$^{b)}$
2	138.3$^{e)}$	138.2$^{e)}$	141.7$^{e)}$	138.3$^{e)}$	137.6	138.2$^{e)}$
3	50.5$^{f)}$	50.3$^{f)}$	59.1	51.2	50.1$^{f)}$	50.2$^{f)}$
5	53.0$^{f)}$	53.0$^{f)}$	59.1	51.2	59.7$^{f)}$	60.6$^{f)}$
6	23.4	23.2	70.6	27.9	26.1	26.8
7	106.0	105.8	102.7	104.1	104.6	103.7
8	$^{d)}$	$^{d)}$	120.5	123.0	122.3	123.1
9	118.8	118.8	118.3	118.7	118.5	118.7
10	108.7	108.7	108.4	108.7	108.7	108.7
11	156.3	156.4	155.3	156.4	156.0	156.2
12	95.8	96.0	95.4	96.0	95.4	96.0
13	137.9$^{e)}$	137.6$^{e)}$	136.6$^{e)}$	137.8$^{e)}$	137.6	137.7$^{e)}$
14	27.9	27.9	27.8	34.4	34.1	36.0
15	27.3	27.9	27.4	28.4	32.5	40.4
16	43.6	43.6	47.9	45.3	36.3	36.2
17	60.5	60.5	63.9	64.7	18.7	19.4
18	13.0	58.0	56.7	13.0	12.6	12.5
19	112.6	120.0	119.5	115.7	116.1	116.0
20	142.3	144.0	141.9	139.2	136.4	139.1
21	56.9	56.6	55.1	56.6	56.3	54.0
O-Me	55.6	55.6	55.5	55.6	55.8	55.6

a) The δ values are in ppm downfield from TMS.
b) Measured in pyridine-d_5.
c) Measured in DMSO-d_6.
d) The corresponding signals are hidden in the peaks of the solvent.
e, f) Signals in any vertical column may be reversed.

16-Epigardnerine (XII)[13] was prepared in three steps from gardnerine (VIII). The CMR spectrum of this compound was in full accord with the structure (XII).

Compound XIII was a novel type of reduction product which was obtained on treatment of gardnutine (X) under Huang–Minlon reduction conditions.[9] The geometrically isomeric compound (XIV) was obtained by Huang–Minlon reduction of the α,β-unsaturated aldehyde (XV) which was derived from hydroxygardnutine by oxidation with MnO_2. The correctness of these structural assignment for XIII and XIV has been confirmed by the present CMR spectral study (Table II), and in particular the configurations of the ethylidene moieties of XIII and XIV are evident from the chemical shifts of the signals due to $C_{(15)}$ and $C_{(21)}$.

Assignment of $C_{(6)}$ and $C_{(14)}$ of the gardnerine type alkaloids (VIII—XIV) was achieved as follows. Of the two methylene carbons observed at 23.4 ppm and 27.9 ppm in the spectrum of VIII, the former was shifted to 70.6 ppm in the spectrum of hydroxygardnutine (XI) which has an oxygen function on $C_{(6)}$. Therefore this peak of VIII and the peak of IX at a similar position (23.2 ppm) should be assigned to $C_{(6)}$. On the other hand, the signal corresponding to those at 27.9 ppm in VIII and IX shifted to lower fields in the spectra of the $C_{(16)}$ epimers XII—XIV. This observation can be reasonably interpreted if these peaks are assigned to $C_{(14)}$ and the change of position is regarded as a result of release from the γ-effect due to the substituent ($C_{(17)}$) on $C_{(16)}$. The same effect was observed to a smaller extent on $C_{(6)}$, consistent with expectation based on examination of a molecular model.

Assignment of the aromatic carbons of the indole nuclei was based on results in the literature for model compounds, such as N_b-methyltetrahydroharmine,[14] reserpine[15] and isoreserpine.[15]

It is interesting to note that in the sarpagine type *Gardneria* alkaloids all natural compounds with or without an oxygen function on $C_{(18)}$ have been confirmed to possess *E* geometry. (*cf.* the discussion on gardneramine type alkaloids described above.)

Experimental

The ^1H noise-decoupled and off-resonance CMR spectra were recorded on a JEOL FX 60 FT-NMR spectrometer operating at 15.04 MHz. The samples were run in 10 mm tubes or micro tubes using $CDCl_3$ (concentrations were about 8% w/v) as the solvent unless otherwise specified, with TMS as an internal reference. FT measurement conditions were as follows: spectra width, 4000 Hz; pulse width, 4 μ sec (45°); repetition rate, 3 sec; data points, 4096.

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13) S. Sakai *et al.*, unpublished results.

14) E. Wenkert, J.B. Bindra, C.-J. Chang, D.W. Cochran, and F.M. Schell, *Accounts Chem. Res.*, **7**, 46 (1974).

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