Tetrahedron Vol. 45, No. 5, pp. 1327 to 1336, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

A BIOMIMETIC CONSTRUCTION OF HUMANTENINE SKELETON

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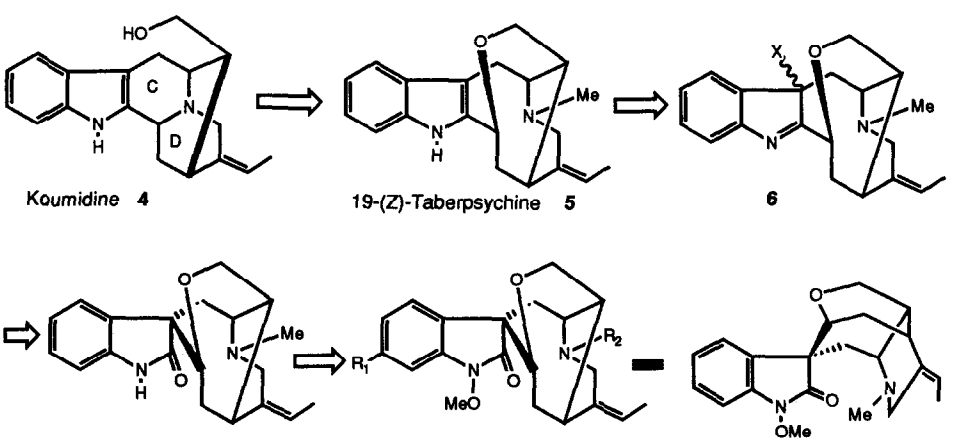
(*Received in Japan* 17 *October* 1988)

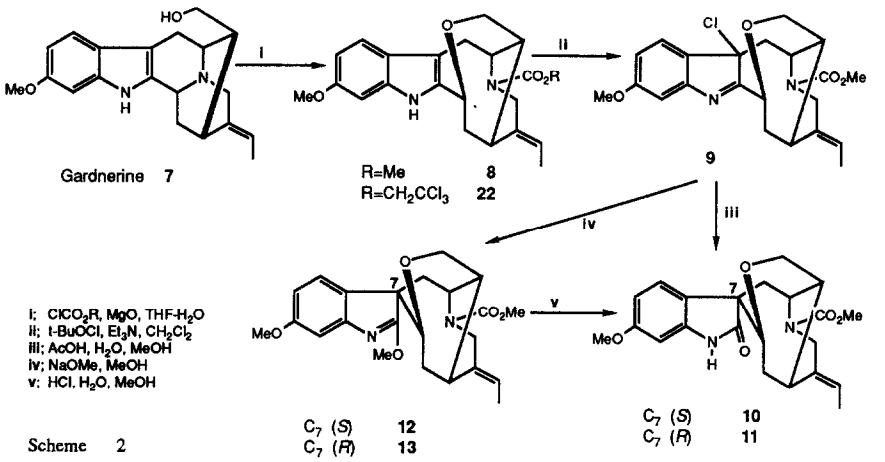
Abstract---The stereoselective transformation of gardnerine (7) into des-N(a)-methoxyhumantenirine (27) (*Gelsemium* alkaloid) is described.

Humantenine-type alkaloids, such as humantenine (1), humantenirine (2), and rankinidine (3) are one of the key members of *Gelsemium* alkaloids, which are well known to contain some various novel type of indole alkaloids.[1] During the chemical studies on *Gelsemium* alkaloids,[2)-5)] we were interested in the partial synthesis of humantenine-type alkaloids from sarpagine class of indole alkaloids, which were possible biogenetic precursor in the plants.[4] As described in Scheme 1, sarpagine-type alkaloid, such as koumidine (4),[4] will be metabolized to a C/D ring opening compound, 19-(Z)-taberpsychine (5).[4] β-Oxidation of indole part in (5) will generate indolenine (6), which will further transform into humantenine-type alkaloids through the rearrangement into oxindole and subsequent methoxylation process of N(a) function. We chose gardnerine (7)[6] as a starting material and designed its chemical conversion along the biomimetic speculation above. The transformation involves mainly three structural changes of the starting material (7): 1) stereoselective preparation of the spiro-oxindole, 2) inversion of the ethylidene side chain, and 3) introduction of the oxygen function onto the nitrogen in oxindole moiety. Here we report the successful accomplishment of the first two requirements, which has enable us the first construction of the humantenine skeleton.

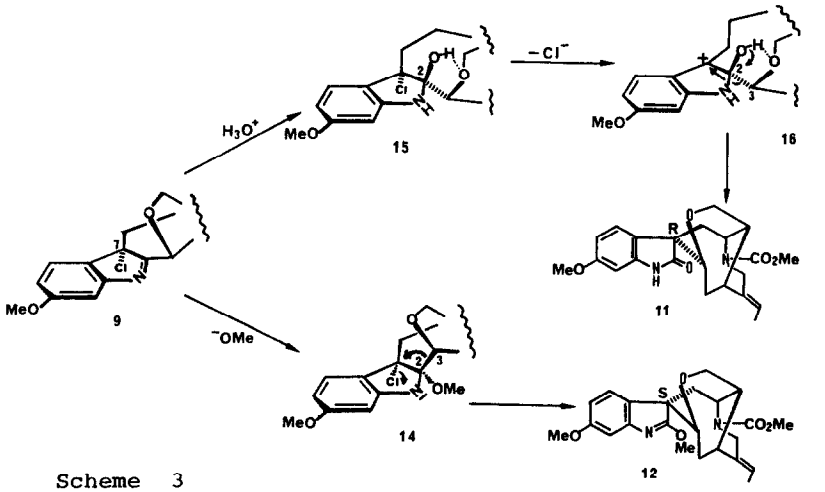


Scheme 1

Little has been studied on the chemical transformation of sarpagine-type indole alkaloids into the corresponding oxindole alkaloids.[7] We initially investigated this crucial step using C/D ring cleavage compound (**8**). (Scheme 2) Treatment of gardnerine (**7**) with 1.3 equiv of methyl chloroformate and large excess of magnesium oxide in aq. THF resulted in the cleavage of the C/D ring and in the simultaneous formation of the intramolecular ether linkage at C_3 position to afford carbamate (**8**) in 90% yield.[8] An usual method for the preparation of oxindole derivative from the corresponding indole compound was initially employed. Thus, oxidation of (**8**) with *t*-butyl hypochlorite in the presence of Et_3N gave the chloroindolenine (**9**). However, (**9**) was so unstable to the usual work up manner that crude reaction products were directly treated with aq. acetic acid solution to afford two oxindoles (**10**) and (**11**) in 9% and 37% yield, respectively. These two products were the diastereomers at C_7 spiro position from their spectroscopies. Furthermore, by the comparison of their CD spectra (Fig. 1) with that of natural humantenine (**1**), the minor product (**10**) had the same absolute configuration at C_7 with that of (**1**). Attempts to epimerize at C_7 in (**10**) in hot acetic acid were unsuccessful. The equilibrium



Scheme 2

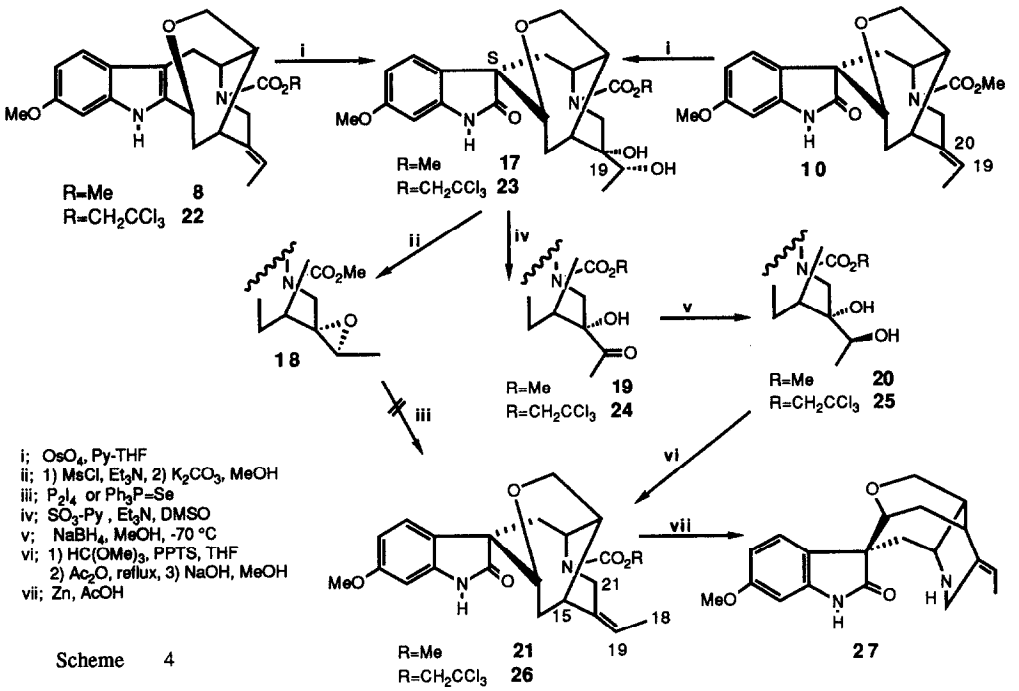


Scheme 3

between spiro-oxindole isomers in *Corynante*- and *Yohimbine*-type alkaloids were usually observed. We next treated the chloroindolenine derivative (**9**) with NaOMe in MeOH solution to give two iminoethers (**12**) and (**13**) in 41% and 23% yield, respectively. From the spectroscopic analysis these two products were the diastereomers at C_7 position and their stereochemistry was determined by the hydrolytic conversion of them into the corresponding oxindoles (**10**) and (**11**), respectively. At this stage we speculated the stereochemical course of the rearrangement reaction, in which the products having opposite configuration at spiro position (C_7) generated predominantly from the common intermediate (**9**) under different two conditions. *t*-BuOCl should approach to β-position of indole part from less hindered side (anti to bridged ether linkage), resulting in the formation of C_7-α-chloroindolenine (**9**). (see Scheme 3) Treated (**9**) with NaOMe, the reagent may attack to C_2 position from α side because of the steric hindrance of the ether linkage to generate the intermediate (**14**), and then the anti-periplanar C_2-C_3 bond to the leaving group (Cl) will rearrange to C_7 position to form (*S*)-isomer (**12**) preferentially. On the other hand, chloroindolenine derivative (**9**) may react with H_2O in aqueous acidic media to form C_2-β-hydroxylated intermediate (**15**) due to the affinity of ether oxygen atom to H_2O. After the formation of carbocation on C_7 by the elimination of Cl^- under S_N1 condition, C_2-C_3 bond in the intermediate (**16**) will move to C_7 position from α-side to form (*R*)-isomer (**11**) predominantly. From the mechanistic consideration above, the preparation of an intermediate such as (**14**) in which both the leaving group on C_7 and the oxygen function on C_2 possesse α-orientation should provide the C_7-(*S*) spiro-isomer in a highly diastereoselective manner. As anticipated, treatment of (**8**) with 2.0 equiv of osmium tetraoxide in pyridine-THF afforded oxindole (**17**) as a sole product in 77% yield. The stereochemistry at C_7 in (**17**) was determined by the correlation to the osmylation product of (**10**). (Scheme 4) From the Dreiding model analysis, OsO_4 may attack to C_{19}-C_{20} double bond from less hindered side to yield the vicinal diol having (*R*) and (*S*) configurations on C_{19} and C_{20}, respectively. It is interesting to note that all of the sarpagine class of oxindole alkaloids from *Gelsemium* spp. (humantenine- and gelsedine-type alkaloids) possessed (*S*)-configuration at spiro position. Oxidative rearrangement of indole alkaloids into the oxindole derivatives in the *Gelsemium* plant may occur enzymatically *via* an intermediate which is similar to that of osmylation process described above.

Next we turned our attention to the olefin inversion utilizing the vicinal diol function obtained by the osmylation reaction above. (Scheme 4) Epoxy derivative (**18**) was derived from (**17**) by two steps (1. MsCl, Et_3N. 2. K_2CO_3-MeOH) accompanied by the inversion at C_{19} configuration. However, all attempts at conversion of the epoxide (**18**) into the olefinic compound did not give satisfactory results. Then we employed Ando's method for the synthesis of olefins from vicinal diols[9]. The configuration at C_{19} in (**17**) was initially inverted by the oxidation-reduction sequence. Thus, (**17**) was oxidized with SO_3-pyridine complex to afford C_{19}-keto derivative (**19**), which was reduced with $NaBH_4$ in MeOH at -75°C to give the desired C_{19}-(*S*) alcohol (**20**) predominantly (**20**:**17**=16:1). Attempts at the direct conversion on C_{19} configuration by means of Mitsunobu procedure or by using CsOAc were unsuccessful. A vicinal diol in (**20**) was converted into the corresponding 2-methoxy-1,3-dioxolane by treatment with trimethyl orthoformate in the presence of pyridinium p-toluenesulfonate in THF. The resulting 2-methoxy-1,3-dioxolane was refluxed in acetic anhydride and then N(a)-acetyl group formed during the former reaction was removed by alkaline hydrolysis to produce the desired olefinic compound (**21**) in 60% yield from (**20**). The configuration of the ethylidene side chain in (**21**) was confirmed by the ^1H- and ^{13}C-NMR analysis. In the ^{13}C-NMR spectra, the signal due to C_{15} of (**21**) was observed at downfield (Δ4.0, 3.5 ppm)[10] and , on the contrary, that of C_{21} was observed at upfield (Δ6.2, 6.6 ppm)[10] than the corresponding signals of (**17**). In the ^1H-NMR spectrum of (**21**), 6% and 8% enhancement were observed in difference NOE experiments between C_{21}-H and C_{18} methyl group and between C_{15}-H and C_{19}-H, respectively.

Frustrated by the efforts (Me_3SiCl/NaI in MeCN, MeLi/THF, KOH/ethylene glycohol) to cleanly remove the methyl carbamate in (**21**), we displaced the protecting group on N(b) and undertook again the chemical conversion of (**7**) along the line developed above. Thus, gardnerine (**7**) was treated with β,β,β-trichloroethyl chloroformate to afford the C/D ring-opening compound (**22**) in 88% yield. The oxidation of (**22**) with 2.0 equiv of OsO_4 in THF-Py. led to oxindole (**23**) as a single isomer in 78% yield. The secondary alcohol on C_{19} in (**23**) was inverted through the oxidation (SO_3-Py., Et_3N in DMSO)-reduction ($NaBH_4$ in MeOH, -78°C) sequence.



Scheme 4

CD Curves of 1, 10, and 11

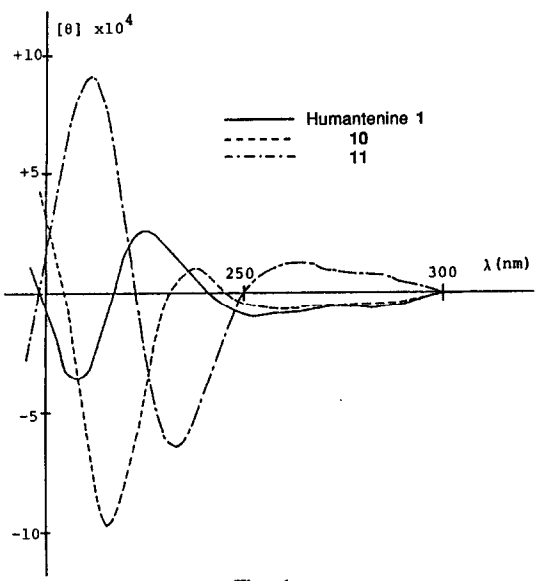


Fig. 1

CD curves of 27 and 28

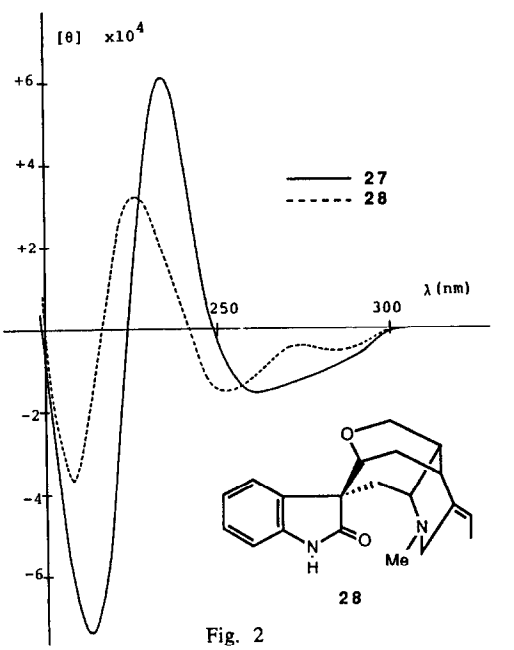


Fig. 2

The olefinic compound having 19-(Z) configuration was obtained in 73% over all yield by the successive treatment of (**25**) with $HC(OMe)_3$/PPTS in THF, Ac_2O reflux, and then NaOH in MeOH. The configuration of the ethylidene side chain in (**26**) was confirmed by the difference NOE experiments. Thus, irradiation of C_{21}-H and C_{15}-H enhanced C_{18}-H_3 and C_{19}-H with 6% and 8% NOE, respectively. Finally the protecting group on N(b) was removed with Zn in AcOH to give the desired des-N(a)-methoxyhumantenirine (**27**) (mp. 262°C). A correlation of CD spectra (Fig. 2) of (**27**) with corresponding data for (**28**), which was derived from humantenine (**1**) by the demethoxylation reaction[11], allowed unambiguous assignments to the structure of (**27**).

The third requirement of this work was methoxylation of nitrogen in the oxindole moiety. All the attempts to introduce an oxygen function onto the secondary amide group by means of hitherto known procedure[12] were unsuccessful. The development of new oxidation method of the lactam function was strongly required and a research along this line is under way in our laboratory.

In conclusion, we succeeded for the first time the construction of humantenine-skeleton along the biogenetic route in a highly stereoselective manner.

Experimental

All melting points were measured on a Yamato MP-21 apparatus and are uncorrected. IR spectra were measured with a Hitachi 260 spectrophotometer, and UV spectra were measured in EtOH with a Hitachi U3400 spectrophotometers. ^{1}H-NMR spectra were recorded on JEOL JNM FX-270 and JNM GX-270 (270 MHz) spectrometers with tetramethylsilane as an internal standard in $CDCl_3$. ^{13}C-NMR spectra were measured with JEOL JNM FX-270 and JNM GX-270 (67.8 MHz) spectrometers with tetramethylsilane as an internal standard. Mass were taken with Hitachi RMU-6E and RMU-7M spectrometers. CD spectra were measured with JASCO J-500A in MeOH. Thin layer chromatography was performed on Merck precoated Silica gel 60F-254 plates. Column chromatography utilized Merck Silica gel 60 [70-230 and 230-400 mesh (for flash chromatography)] and pre-packed column [Kusano CPS-223L-1 and CPS-HS-221-05 (for medium pressure column chromatography)]. Abbreviations used are: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), shoulder (sh).

Preparation of carbamate (**8**) from gardnerine (**7**)

To a stirred mixture of gardnerine (900mg, 2.78mmol) and MgO (2.72g, 67.5mmol) in THF (90ml) and H_2O (36ml) was added $ClCO_2Me$ (0.27ml, 3.48mmol) at O°C and the mixture was stirred at rt for 50min under argon atmosphere. The reaction mixture was filtered and the filtrate was concentrated and then acidified with 1N-HCl solution. The whole was extracted with $CHCl_3$. The organic extract was washed with H_2O, dried over Na_2SO_4 and evaporated. The residue was purified by flash column chromatography (SiO_2, 15% AcOEt/benzene) to afford 927mg (y. 87%) of (**8**) as an amorphous solid. IR($CHCl_3$) 3450, 1685, 1630, 1460 cm^{-1}; UV(EtOH) 297, 279, 235 nm; ^{1}H-NMR δ 7.48(1H, d, J=8.6Hz), 6.81(1H, d, J=8.6Hz), 6.77(1H, s), 5.48(1H, m, C_{19}-H), 5.14(1H, d, J=9.6Hz, C_3-H), 3.82(3H, s, OMe), 3.74 and 3.75(3H, each s, COOMe)[10], 1.69(1H, d-like, J=5.3Hz, C_{18}-H); ms m/z(%) 382(M^+, 100), 307(11), 257(11), 236(22), 186(54), 166(27), 165(27), 111(29).

Preparation of oxindoles (**10**) and (**11**) from carbamate (**8**)

To a solution of (**8**) (350mg, 0.92mmol) and Et_3N (180μl) in dry CH_2Cl_2 (18ml) was added a 0.658×10^{-4} mol solution of *t*-BuOCl in CCl_4 (14ml, 0.92mmol) dropwise under argon atmosphere at -15°C and the mixture was stirred at the same temperature for 1h. Solvent was removed under reduced pressure at 0°C and the residue was immediately treated with the mixture of MeOH, H_2O and AcOH (40:20:1, 7ml) under reflux for 1h. The reaction mixture was basified with aq NH_4OH solution and then extracted with $CHCl_3$. The organic extract was washed with H_2O, dried over Na_2SO_4 and evaporated. The oily residue was separated by medium pressure liquid chromatography (MPLC) (SiO_2, 50% n-hexane/AcOEt) to afford 31mg (y. 9%) of (**10**) as an amorphous powder and 135mg (y. 37%) of (**11**) as colorless needles. (**10**): IR($CHCl_3$) 3400, 2950, 1700, 1630, 1450 cm^{-1}; UV(EtOH) 292(sh), 285(sh),

256, 215 nm; ^{1}H-NMR δ 5.58(1H, q, J=6.7Hz, C_{19}-H), 4.23-4.04(4H, m, C_{21}-H, C_{17}-H), 3.80(3H, s, OMe), 3.68(3H, s, CO_2Me), 3.66(1H, d, J=10.1Hz, C_3-H), 1.73(3H, d, J=6.7Hz, C_{18}-H); ms m/z(%) 398(M^+, 100), 223(43), 222(59), 194(63); ^{13}C-NMR ppm 180.8(s, C_2), 72.4(d, C_3), 54.7(d, C_5), 34.0 and 33.3(each t, C_6)[10], 56.3(s, C_7), 137.0(s, C_8), 127.1(d, C_9), 107.1(d, C_{10}), 155.4(s, C_{11}), 97.3(d, C_{12}), 141.1(s, C_{13}), 29.3(t, C_{14}), 28.5(d, C_{15}), 37.6(d, C_{16}), 66.3(t, C_{17}), 14.1(q, C_{18}), 122.0(d, C_{19}), 124.3(s, C_{20}), 45.7(t, C_{21}), 55.5(q, COOMe), 160.1(s, CO), 52.5(q, OMe); CD(c=0.27 mmol/l, MeOH, 23.5°C): [θ]3100, [θ]280-5185, [θ]260-6300, [θ]2450, [θ]237+10370, [θ]2310, [θ]226-96290, [θ]2040. (**11**): mp. 248-250°C (acetone); IR(KBr) 3400, 2950, 1720, 1680, 1630, 1460 cm^{-1}; UV(EtOH) 294(sh), 287(sh), 260, 216 nm; ^{1}H-NMR δ 5.59(1H, d, J=7.0Hz, C_{19}-H), 4.58(1H, d, J=10.4Hz, C_{17}-H), 3.96(1H, dd, J_1=10.4, J_2=5.5Hz, C_{17}-H), 4.12(2H, s, C_{21}-H), 3.78(1H, d, J=7.3Hz, C_3-H), 3.76(3H, s, OMe), 3.68(3H, s, CO_2Me), 1.72(3H, d, J=7.0Hz, C_{18}-H); ms m/z(%) 398(M^+, 74), 223(49), 222(75), 194(50), 176(88), 175(100), 132(52); Anal. calcd. $C_{22}H_{26}N_2O_5$: C=66.31, H=6.58, N=7.03, Found: C=66.04, H=6.53, N=6.91; CD(c=0.25 mmol/l, MeOH, 23.5°C): [θ]3100, [θ]285+7200, [θ]264+11200, [θ]2500, [θ]233-63200, [θ]2230, [θ]221+89600.

CD spectrum of humantenine (**1**) (c=0.29mmol/l, MeOH, 23°C): [θ]3250, [θ]282-6200, [θ]274-5650, [θ]252-9100, [θ]2410, [θ]225+26200, [θ]2170, [θ]208-35200.

Preparation of imino ethers (**12**) and (**13**) from (**8**)

To a stirred mixture of (**8**) (200mg, 0.52mmol) and Et_3N (96 μl) in CH_2Cl_2 (8ml) cooled at -10°C was added *t*-BuOCl (62ml) dropwise manner. After 45 min the solvent was removed under reduced pressure at 0°C and the residue was immediately treated with 1N NaOMe-MeOH solution (7ml) for 1h under reflux condition. After concentration of methanol, water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic layer was washed with water, dried over Na_2SO_4, and evaporated. The residue was purified by MPLC (SiO_2, 50% n-hexane/AcOEt) to afford 89mg (y. 41%) of (**12**) and 49mg (y. 23%) of (**13**). (**12**): amorphous powder, IR($CHCl_3$) 1680, 1580, 1450 cm^{-1}; UV(EtOH) 295(sh), 284(sh), 267(sh), 226(sh), 221 nm; ^{1}H-NMR δ 4.00(3H, s, C_2-OMe), 3.80(3H, s, C_{11}-OMe), 3.66(3H, s, CO_2Me), 3.57(1H, d, J=6.1Hz, C_3-H), 1.73(3H, d, J=6.7Hz, C_{18}-H); ms m/z(%) 412(M^+, 86), 223(55), 222(38), 190(100), 189(41). (**13**): amorphous powder, IR($CHCl_3$) 1690, 1575, 1450 cm^{-1}; UV(EtOH) 295(sh), 284(sh), 266(sh), 226(sh), 221 nm; ^{1}H-NMR δ 5.62(1H, m, C_{19}-H), 4.13 and 4.08(3H, each s, C_2-OMe)[10], 3.79(3H, s, OMe), 3.68(3H, s, COOMe), 1.75(3H, d, J=6.6Hz, C_{18}-H); ms m/z(%) 412(M^+,45), 223(27), 222(19), 190(100), 189(17).

Hydrolysis of imino ether (**12**)

A mixture of (**12**) (80mg, 0.194mmol), conc HCl (1.6ml), H_2O (4ml) and MeOH (4ml) was heated under reflux for 1h. The reaction mixture was diluted with ice-water and then basified with aq NH_4OH solution. The whole was extracted with $CHCl_3$ and the organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by MPLC (SiO_2, 50% n-hexane/AcOEt) to afford 72mg (y. 94%) of (**10**), which was identical with the authentic sample prepared from (**8**) by the comparison of their ^{1}H-NMR and TLC behavior.

Hydrolysis of imino ether (**13**)

Same treatment of (**13**) (10mg, 0.024mmol) with conc.HCl, H_2O and MeOH (1:15:15, total 1ml) afforded 7mg (y. 68%) of (**11**), which was identical with authentic sample by the comparison of their ^{1}H-NMR and TLC behavior.

Osmylation of indole derivative (**8**)

To a stirred solution of (**8**) (27mg, 0.071mmol) in dry THF (0.7ml) and dry pyridine (0.7ml) was added OsO_4 (37mg, 2.0equiv) and the mixture was stirred at rt for 3h. Aqueous $NaHSO_3$ solution (75mg in 0.5ml H_2O) was added and the mixture was stirred for 3.5h. After the addition of ice-water the reaction mixture was basified with 10% Na_2CO_3 solution and then the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by MPLC eluted with AcOEt to give 23mg (y. 77%) of (**17**) as an amorphous powder, which was homogeneous on HPLC analysis. IR($CHCl_3$) 3400, 3020, 1700, 1630, 1460 cm^{-1}; UV(EtOH) 292(sh), 285(sh), 260, 216 nm; ^{1}H-NMR δ 3.79(3H, s, OMe), 3.69(3H, s, COOMe), 1.25(3H, d, J=6.1Hz, C_{18}-H); ms m/z(%) 432(M^+, 100), 256(70), 176(78), 175(86).

Osmylation of oxindole derivative (**10**)

To a solution of (**10**) (30mg, 0.075mmol) in dry THF (0.5ml) and dry pyridine (0.5ml) was added OsO_4 (20.5mg, 1.1equiv) and the mixture was stirred at rt for 3h. Aqueous $NaHSO_3$ solution (78mg in 1ml H_2O) was added and the mixture was stirred for 5h. After the addition of ice-water the reaction mixture was basified with 10% Na_2CO_3 solution and then the whole was extracted with $CHCl_3$. The organic extract was washed with brine, dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel eluted with AcOEt to afford 30mg (y. 92%) of (**17**), which contained 18% of a diastereoisomer with respect to C_{19}-C_{20} position. The major diastereomer (**17**) was identical with authentic (**17**) derived from (**8**) by the comparison of ^{1}H-NMR and TLC behavior.

Oxidation of alcohol (**17**)

A solution of SO_3-pyridine complex (221mg, 3.0equiv) in dry DMSO (1ml) was added dropwise to a stirred mixture of (**17**) (200mg 0.463mmol) and Et_3N (0.65ml) in dry DMSO (0.5ml). After 30min water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic extract was washed with water, dried over $MgSO_4$ and evaporated. DMSO was removed by Kugel rohr apparatus under reduced pressure and the residue was purified by MPLC (AcOEt) to afford 161mg (y. 81%) of (**19**) as an amorphous powder. IR($CHCl_3$) 3420, 3000, 1710, 1630, 1460 cm^{-1}; UV(EtOH) 292(sh), 285(sh), 259, 216 nm; ^{1}H-NMR δ 2.34(3H, s, C_{18}-H); ms m/z(%) 430(M^+, 100), 254(48), 176(84), 175(77).

$NaBH_4$ reduction of keto derivative (**19**)

To a solution of (**19**) (56mg, 0.13mmol) in methanol (2ml) cooled at -75°C was added a solution of $NaBH_4$ (50mg) in methanol (1ml) and the mixture was stirred at the same temperature for 1h. Water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was separated by MPLC (3% MeOH/AcOEt) to give 46mg (y. 82%) of (**20**) and 3mg (y. 5%) of (**17**). (**20**): IR($CHCl_3$) 3425, 1700, 1630, 1460 cm^{-1}; UV(EtOH) 292(sh), 285(sh), 260, 216 nm; ^{1}H-NMR δ 3.80(3H, s, OMe), 3.69(3H, s, CO_2Me), 1.27(3H, d, J=6.1Hz, C_{18}-H); ms m/z(%) 432(M^+, 83), 256(80), 176(93), 175(100).

Preparation of olefinic compound (**21**) from diol (**20**)

To a solution of (**20**) (50mg, 0.116mmol) in dry THF (1ml) was added pyridinium p-toluenesulfonate (15mg, 0.5equiv) and trimethyl orthoformate (64μl, 5equiv) and the mixture was stirred at rt for 2h. The solution was passed through a short column of silica gel (2g) and concentrated. The resulting 1,3-dioxolane was dissolved in acetic anhydride (1ml) and the solution was refluxed under argon for 2h. 10% Na_2CO_3 solution was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was dissolved in 1N NaOMe/MeOH (1ml) solution and the mixture was stirred for 30min at rt. Water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic extract was washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by MPLC (50% n-hexane/AcOEt) to afford 25mg (y. 60%) of (**21**) as an amorphous powder. IR($CHCl_3$) 3440, 1715, 1690, 1640, 1460 cm^{-1}; UV(EtOH) 292(sh),

284(sh), 256, 215 nm; ^{1}H-NMR δ 5.61(1H, q, J=5.5Hz, C_{19}-H), 4.39 and 4.33 (1H, each d, J=15.6Hz, C_{21}-H)[10], 4.21-4.04(3H, m, C_{17}-H, C_{21}-H), 3.81 and 3.79(3H, each s, OMe)[10], 3.70(3H, s, CO_2Me), 3.68(1H, d, J=13.4Hz, C_3-H), 2.79(1H, m, C_{15}-H), 1.74 and 1.72 (3H, each d, J=5.5Hz, C_{18}-H)[10]; ms m/z(%) 398(M^+, 100), 223(48), 222(58), 194(55), 176(62), 175(53); ^{13}C-NMR ppm 180.9(s, C_2), 72.5(d, C_3), 55.0 and 54.5(each d, C_5)[10], 33.8 and 33.1(each t, C_6)[10], 56.4(s, C_7), 136.8(s, C_8), 127.0(d, C_9), 106.9(d, C_{10}), 155.6(s, C_{11}), 97.2(d, C_{12}), 140.9(s, C_{13}), 29.9(t, C_{14}), 32.5 and 32.0(each d, C_{15})[10], 37.7(d, C_{16}), 66.1(t, C_{17}), 13.3(q, C_{18}), 122.0(d, C_{19}), 124.3(s, C_{20}), 39.5 and 39.1(each t, C_{21})[10], 55.5(q, CO_2Me), 160.0(s, CO), 52.6(q, OMe).

Preparation of carbamate (22) from gardnerine (7)

To a stirred mixture of gardnerine (900mg, 2.78mmol) and MgO (2.72g, 67.5mmol) in THF (90ml) and H_2O (36ml) was added $ClCO_2CH_2CCl_3$ (0.48ml, 3.49mmol) at 0°C and the mixture was stirred at rt for 30min under argon atmosphere. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure and then acidified with 1N-HCl solution. The aqueous layer was extracted with $CHCl_3$ and the organic extract was washed with water, dried over Na_2SO_4 and evaporated. The residue was purified by flash column chromatography (SiO_2, 50% n-hexane/AcOEt) to give 1.221g (y. 88%) of (**22**) as an amorphous powder. IR($CHCl_3$) 3440, 1690, 1620, 1410 cm^{-1}; UV(EtOH) 297, 278, 224.5 nm; ^{1}H-NMR δ 5.52(1H, m, C_{19}-H), 5.16(1H, d, J=10.1Hz, C_3-H), 5.06 and 4.59(each d, J=11.9Hz, $COOCH_2CCl_3$)[10], 4.81 and 4.77(each d, J=11.9Hz, $COOCH_2CCl_3$)[10], 3.83(3H, s, OMe), 1.71(3H, dt, J=6.7, 1.8Hz, C_{18}-H); ms m/z(%) 500(M^++2, 80), 498(M^+, 89), 466(58), 464(100), 448(21), 446(24), 351(43), 323(28), 289(28), 276(28), 266(27), 252(28), 248(29), 238(36), 236(39), 224(41), 211(43), 210(93), 198(65), 186(62); High Resolution ms Calcd for $C_{23}H_{25}N_2O_4Cl_3$: 498.0878, Found: 498.0857.

Preparation of oxindole (23) from carbamate (22)

To a stirred solution of (**22**) (223mg, 0.446mmol) in dry THF (1.5ml) and dry pyridine (1.5ml) was added OsO_4 (228mg, 2.0equiv) and the mixture was stirred at rt for 1h. Aqueous $NaHSO_3$ solution (400mg in 1ml H_2O) was added and the mixture was stirred for 4h. After the addition of ice-water the reaction mixture was basified with 10% Na_2CO_3 solution and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by MPLC (5% MeOH/$CHCl_3$) to afford 192mg (y. 78%) of (**23**). IR($CHCl_3$) 3430, 1710, 1630, 1120 cm^{-1}; UV(EtOH) 292(sh), 285(sh), 258, 216 nm; ^{1}H-NMR δ 7.73 and 7.71(1H, each br-s, $N_{(a)}$-H)[10], 4.88 and 4.64(each 1H, d, J=11.9Hz, $COOCH_2CCl_3$), 3.80(3H, s, OMe), 3.63(1H, d, J=7.3Hz, C_3-H), 1.27 and 1.26 (3H, each d, J=5.9Hz, C_{18}-H)[10]; ms m/z(%) 550(M^++2, 12), 548(M^+, 13), 176(100), 175(77).

Oxidation of alcohol (23)

A solution of SO_3-pyridine complex (78mg, 0.490mmol) in dry DMSO (0.5ml) was added dropwise to a stirred mixture of (**23**) (90mg, 0.164mmol) and Et_3N (230μl) in dry DMSO (0.5ml). After 1h water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic extract was washed with water, dried over $MgSO_4$ and evaporated. DMSO was removed by Kugel rohr apparatus and the residue was purified by MPLC (30% n-hexane/AcOEt) to give 55mg (y. 61%) of (**24**) as an amorphous powder. IR($CHCl_3$) 3425, 1705, 1630, 1150 cm^{-1}; UV(EtOH) 292(sh), 285(sh), 258, 216 nm; ^{1}H-NMR δ 4.86 and 4.65(each 1H, d, J=11.9Hz, $COOCH_2CCl_3$), 2.35 and 2.34(3H, each s, C_{18}-H)[10]; ms m/z(%) 548(M^++2, 12), 546(M^+, 13), 176(100), 175(66).

NaBH4 reduction of keto derivative (24)

To a solution of (**24**) (301mg, 0.550mmol) in methanol (2ml) cooled at -78°C was added dropwise a solution of $NaBH_4$ (280mg, 7.40mmol) in methanol (3ml) and the mixture was stirred at the same temperature for 1h. Water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was

separated by MPLC (20% n-hexane/AcOEt) to yield 193mg (y. 64%) of (**25**) and 12mg (y. 4%) of (**23**). (**25**): IR($CHCl_3$) 3430, 1705, 1630, 1120 cm^{-1}; UV(EtOH) 292(sh), 285(sh), 257, 216 nm; ^{1}H-NMR δ 7.64 and 7.61(1H, each br-s, $N_{(a)}$-H)[10], 4.80 and 4.70(each 1H, d, J=11.9Hz, $COOCH_2CCl_3$), 3.80(3H, s, OMe), 3.67(1H, d, J=6.7Hz, C_3-H), 1.28 and 1.26 (3H, each d, J=5.5Hz, C_{18}-H)[10]; ms m/z(%) 550(M^++2, 19), 548(M^+, 20), 176(100), 175(82).

Preparation of olefinic compound (**26**) from diol (**25**)

To a solution of (**25**) (190mg, 0.346mmol) in dry THF (2ml) was added pyridinium p-toluenesulfonate (44mg, 0.175mmol) and trimethyl orthoformate (190μl, 1.73mmol) and the mixture was stirred at rt for 2h. The solution was passed through a short column of silica gel (1.5g) and concentrated. The resulting 1,3-dioxolane was dissolved in acetic anhydride (2ml) and the solution was refluxed under argon for 2h. After the addition of ice-water the reaction mixture was basified with aq NH_4OH solution and the whole was extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was dissolved in 2.5N NaOH/MeOH solution (3ml) and the mixture was stirred at rt for 30min. Water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The organic extract was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by MPLC (50% n-hexane/AcOEt) to give 130mg (y. 73%) of (**26**). IR($CHCl_3$) 3440, 3000, 1710, 1635, 1420; UV(EtOH) 292(sh), 285(sh), 257, 215; ^{1}H-NMR δ 7.78 and 7.75(1H, each br-s, $N_{(a)}$-H)[10], 5.69-5.62(1H, m, C_{19}-H), 4.72(2H, d, J=13.2Hz, $COOCH_2CCl_3$), 4.50 and 4.48(1H, each d, J=15.6Hz, C_{21}-H)[10], 4.28-3.99(3H, m, C_{17}-H_2, C_{21}-H), 3.79(3H, s, OMe), 3.67(1H, dd, J=11.0, 5.5Hz, C_3-H), 2.84(1H, m, C_{15}-H), 1.76 and 1.74(3H, each d, J=6.7Hz, C_{18}-H)[10]; ms m/z(%) 516(M^++2, 32), 514(M^+, 35), 176(100), 175(70).

Des-N(a)-methoxyhumantenirine (**27**)

To a solution of (**26**) (33mg, 0.064mmol) in acetic acid (1ml) was added Zn dust (130mg) and the mixture was stirred at rt for 1h. The reaction mixture was filtered and the filtrate was concentrated. The mixture was basified with aq NH_4OH solution and the whole was extracted with 5% MeOH/$CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was crystallized from MeOH/AcOEt to afford 12mg (y. 55%) of (**27**) as colorless needles. mp. 262°C, IR(KBr) 3280, 2920, 1700, 1640, 1510 cm^{-1}; UV(EtOH) 292(sh), 285(sh), 261, 216 nm; ^{1}H-NMR δ 7.8(1H br-s, $N_{(a)}$-H), 7.29(1H, d, J=8.3Hz, C9-H), 6.59(1H, dd, J=8.3, 2.4Hz, C_{10}-H), 6.42(1H, d, J=2.4Hz, C_{12}-H), 5.23(1H, q, J=7.0Hz, C_{19}-H), 4.30(1H, d, J=10.4Hz, C_{17}-H), 4.03(1H, dd, J=10.4, 4.6Hz, C_{17}-H), 3.87(1H, d, J=16.5Hz, C_{21}-H), 3.79(3H, s, OMe), 3.56(1H, d, J=8.6Hz, C_3-H), 3.32(1H, d, J=16.5Hz, C_{21}-H), 2.63-2.56(1H, m, C_{15}-H), 1.59(3H, d, J=7.0Hz, C_{18}-H); ms m/z(%) 340(M^+, 100), 165(50), 164(70), 108(77); Anal. Calcd for $C_{20}H_{24}N_2O_3 \cdot 1/3H_2O$: C=69.34, H=7.18, N=8.08, Found: C=69.30, H=7.08, N=7.74; CD(c=0.31 mmol/l, MeOH, 25°C): $[\theta]_{3150}$, $[\theta]_{261}$-15220, $[\theta]_{249}0$, $[\theta]_{234}$+58070, $[\theta]_{224}0$, $[\theta]_{213}$-72910.

Des-N(a)-methoxylation of humantenine (**1**)

A solution of humantenine (**1**) (20mg, 0.056mmol) in dry MeOH (0.1ml) was added to a stirred mixture of lithium (5mg) in liquid ammonia (1.5ml) at -78°C and the reaction mixture was stirred at the same temperature for 3min. To quench the reaction saturated aqueous NH_4Cl solution was added and the whole was extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by MPLC (10% MeOH/$CHCl_3$) to yield 13mg (y. 72%) of des-N(a)-methoxyhumantenine (**28**) as colorless needles. mp. 210-211°C, IR(KBr) 3250, 1700, 1620, 1475, 1205, 1115 cm^{-1} ; UV(EtOH) 293(sh), 282(sh), 250, 207; ^{1}H-NMR δ 7.92(1H, br-s, $N_{(a)}$-H), 5.38(1H, q, J=6.7Hz, C_{19}-H), 4.23(1H, d, J=11.0Hz, C_{17}-H), 4.07(1H, dd, J=11.0, 5.2Hz, C_{17}-H), 3.65(1H, d, J=7.3Hz, C_3-H), 3.39(2H, s, C_{21}-H), 2.66-2.57(1H, m, C_{15}-H), 2.37(3H, s, N-Me), 1.65(3H, d, J=6.7Hz, C_{18}-H); ms m/z(%) 324(M^+, 92), 309(100), 122(66); High resolution ms Calcd for $C_{20}H_{24}N_2O_2$:

324.1838, Found: 324.1837 ;CD(c=0.34 mmol/l, MeOH, 25°C): $[\theta]_{315}$0, $[\theta]_{284}$-5410, $[\theta]_{274}$-4350, $[\theta]_{253}$-14700, $[\theta]_{242}$0, $[\theta]_{226}$+31760, $[\theta]_{217}$0, $[\theta]_{208}$-35880.

Acknowledgments

The authors are grateful to the Ministry of Education, Science and Culture of Japan for financial support [Grant-in-Aid for scientific Research (No. 62771832)] of this research. The authors wish to thank the members of Chemical Analysis Center of Chiba University for the measurement of NMR, MS and elemental analysis.

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