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Structure and Synthesis of a New Indole Alkaloid, 19(*S*)-Hydroxy-N_b-methylraumacline, Obtained by the Biotransformation of Ajmaline in Plant Cell Cultures of *Rauwolfia Serpentina* Benth.

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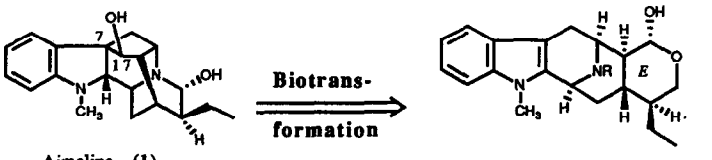
Abstract: From the plant cell suspension cultures of *Rauwolfia serpentina* Benth., which were cultivated in the alkaloid-production medium after feeding of ajmaline (**1**), a new indole alkaloid 19-(*S*)-hydroxy-N_b-methylraumacline (**4**) was isolated. The structure of **4** first elucidated by spectroscopic analysis was determined by the chemical synthesis from ajmaline (**1**).

The Indian medicinal plant *Rauwolfia serpentina* Benth. has been playing an important role for several decades as a rich source of the useful medicines. The cell suspension culture of this plant produces many sarpagan and ajmalan type of indole alkaloids, in which ajmaline (**1**) is accumulated as a main secondary metabolite.[1] In the cultivated *Rauwolfia* cells, ajmaline (**1**) was considered to be a biosynthetic end product and its biogenetic pathway was extensively investigated.[2] During this research we have recently found that after feeding of ajmaline it was further metabolized by the cultivated cells to yield the new type of indole alkaloids, raumacline (**2**) and N_b-methylraumacline (**3**)[3] (Figure 1). Together with these main transformation products, the presence of other minor components has been indicated by the preliminary chromatographic analysis of the crude extracts. We describe here the isolation, spectroscopic identification and chemical synthesis of a new minor raumacline class of indole alkaloid obtained by this feeding experiments.

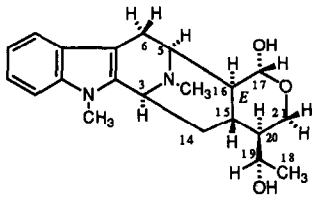


Ajmaline (**1**)

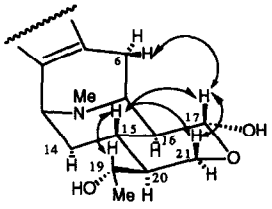
R=H: Raumacline (**2**)
R=Me: N_b-Methylraumacline (**3**)

Fig. 1

Rauwolfia serpentina cell suspensions cultivated for 15 days in the presence of 1g ajmaline/1 medium[3] were extracted with organic solvents. The crude alkaloid fraction was purified by chromatography on silica gel column and finally by preparative TLC to give the new alkaloid (**4**) as an amorphous powder, $[\alpha]_D^{25}$ -86.7° ($CHCl_3$). Its UV absorptions at 223, 282 and 290 nm indicated that it possessed an indole nucleus and the ^{1}H-NMR spectrum showed signals for two *N*-methyl groups, four aromatic protons and a characteristic hemiacetal proton at δ4.55 (d, J=8.8 Hz), which were similar to those of N_b-methylraumacline (**3**),[3] except that **4** had a hydroxyethyl group (δ3.82, 1H, m and δ0.68, 3H, d, J=6.6 Hz) instead of the ethyl group of **3**. The high resolution mass spectrum of the alkaloid displayed a molecular ion at m/z 356.2098 ($C_{21}H_{28}N_2O_3$), 16 mass units more than that of **3**, supporting the notion that **4** might be a hydroxy derivative of **3**. The ^{13}C-NMR spectrum of **4** indicated the presence of eight aromatic carbons due to an indole nucleus, seven aliphatic methine, three methylene and three methyl carbon atoms. Unambiguous assignments of all the carbons and protons were obtained using HH-COSY and CH-COSY technique. The presence of a characteristic *E*-ring of the raumacline-type compounds having a macroline skeleton was demonstrated by the clear relationships observed between the hemiacetal proton (δ4.55) and the 16-H (δ1.74), and between 20-H (δ1.59) and ABX type resonance[δ3.93 (1H, dd, J=11.3, 4.2 Hz), 3.20 (1H, dd, J=11.3, 11.3 Hz)] attached to an oxygen function. A signal at δ3.82 attached to the hydroxy function had the connectivities between the methyl group (δ0.68, d, J=6.6 Hz) and 20-H (δ1.59), then the position of the additional hydroxy group was deduced to be at the C19 position. A signal of 17-H (δ4.55) gave the cross peaks with 21β-H (δ3.20) and 15-H (δ1.24) in the NOESY spectrum, indicating the α-equatorial orientation of the 17-OH group (Figure 2). Intensive interactions between 6β-H (δ2.61, d, J=16.8 Hz) and 17-H, and between 16-H and 14α-H (δ1.71) showed the α-axial configuration of 16-H. The observation of NOE between 19-H and 15-H as well as the large *J* value (11.3 Hz) observed between 21β-H and 20-H indicated the C_{20} (*S*) configuration. From these spectroscopic data the structure of the new alkaloid was deduced to be 19-hydroxy-N_b-methylraumacline.



19(*S*)-Hydroxy-N_b-methylraumacline (**4**)



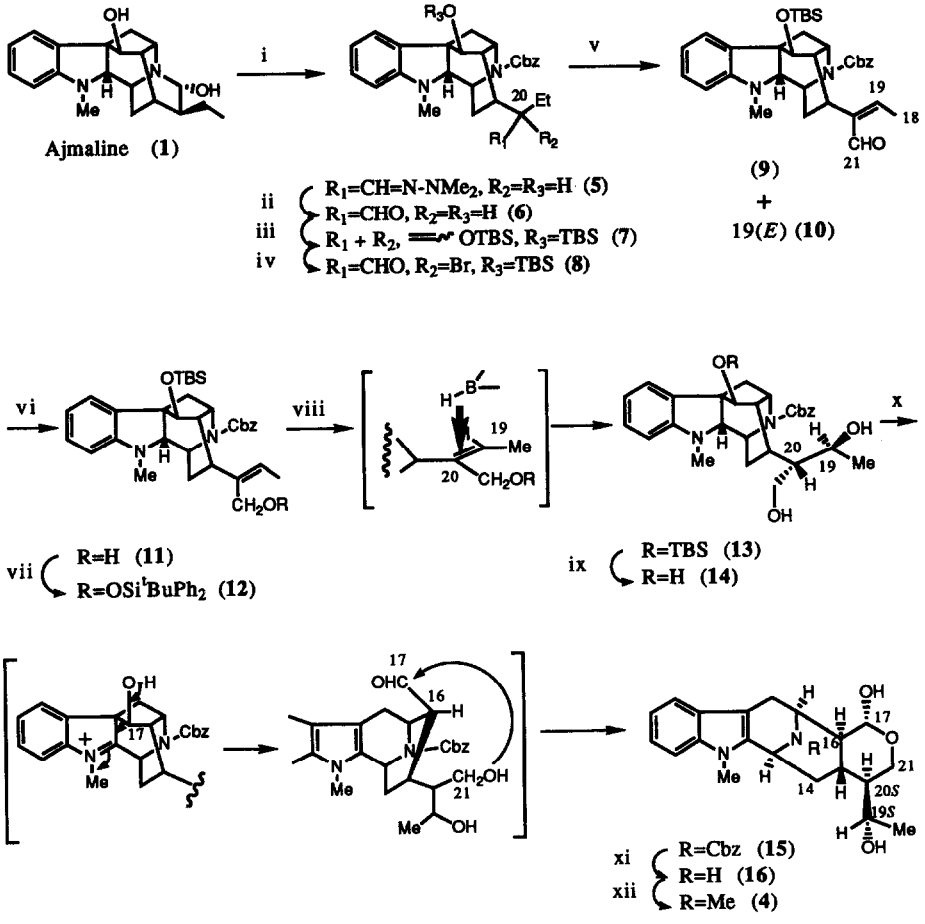
NOE data of **4**

Fig. 2

In order to determine the structure of **4** including the configuration of C19 position, we planned the chemical synthesis of **4** from ajmaline (**1**). To achieve this transformation, the introduction of an hydroxy function to C19 and formation of the macroline skeleton from indoline compound were required. We designed the olefinic compound (**11**) as a synthetic key intermediate, which would offer the 19-hydroxy derivatives by hydroboration. Initially, ajmaline (**1**) was converted to the aldehyde (**6**) *via* the three steps sequence (1. hydrazone formation, 2. N_b protection, 3. hydrolysis of hydrazone).[4] Bromine was then introduced to the C20 position *via* the *t*-

butyldimethylsilyl (TBS) enol ether (7).[5] Treatment of (8) with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF gave two olefinic compounds (9 and 10) in 71% and 21% yield, respectively. The geometry of olefin in the major isomer (9) was determined by NOE experiments. Irradiation of the 18-methyl protons led to enhancement (22%) of the aldehyde proton, while 20% enhancement was observed between the C19 olefinic proton and C21 aldehyde proton in the minor product (10). The allylic alcohol which was obtained in 98% yield by $NaBH_4$ reduction of the aldehyde (9) was protected with *t*-butyldiphenylsilyl group. Hydroboration (boran methylsulfide complex)-oxidation (hydrogene peroxide) of 12 and successive treatment with tetrabutylammonium fluoride (TBAF) for 1 h to cleave the protecting group of the primary alcohol provided two diastereomeric secondary alcohols in 30% and 4% yield, respectively, together with unidentified compounds having an ethyl group in the molecule. These by-products might be produced by the addition of boron to the C20 position in 12.[6] The stereochemistry at the C19 and C20 of the major alcohol (13) was determined by using NOE experiments in the ring-fixed compound 15 (*vide infra*). The protecting group at the C17 in the major product was removed in 96% yield with TBAF at room temperature for 40 h to give the alcohol (14). Oxidation of the indoline moiety in 14 with one equiv. of lead tetraacetate [$Pb(OAc)_4$][7] in dry CH_2Cl_2 at -70°C afforded the macroline skeleton compound 15 in 71% yield *via* the generation of N_a-methylindole and subsequent ring-closure between 21-OH and the resulting aldehyde at C17. The NOE experiments of 15 as well as the mechanistic consideration of hydroboration made clear the stereochemistry at the C19 and C20 positions. Thus, irradiation at H-19 (δ3.80) and 18-methyl protons (δ0.81) showed the 8.2% and 4.9% enhancement of 14β-H (δ1.96) and 21β-H (δ3.24), respectively. This indicates that hydroxyethyl group in 15 is located at β-equatorial position, meaning that C20 has *S* configuration. In order to generate the 20(*S*) isomer from the 19(*Z*) olefin (12) by the hydroboration, BH_3 should approach from (19-*si*, 20-*re*) face. As it is well established that hydroboration proceeds in *cis* addition manner, the configuration at C19 should be *S*. During the transformation from 14 to 15 through the aldehyde intermediate the epimerization at C16 is possible, but strong NOE observed between 6β-H (δ2.82) and the hemiacetal proton at C17 (δ4.54) indicates that the configuration at C16 is retained. Removal of the CBZ group in 15 by catalytic hydrogenolysis and *N*-methylation of the resultant secondary amine (16) with sodium cyanoborohydride in formaline in the presence of catalytic amounts of acetic acid furnished 4 in 72% over all yield from 15. The semisynthetic compound was identical with the natural product by the comparison of their $[\alpha]_D$, chromatographic behavior, MS, ^1H (500 MHz)- and ^{13}C-NMR spectra.

In conclusion, we could find out and identify an additional raumacline type indole alkaloid produced by the biotransformation of ajmaline in plant cell cultures and succeeded in the chemical synthesis of this new compound. We also demonstrated that structurally novel compounds different from the original secondary metabolites were produced by feeding the appropriate substrate to the dedifferentiated plant cells. Therefore, basing on the inherent functions of the cell cultures the creation of new type biologically active compounds could be expected.



Reagents : *i*, *N,N*-Dimethylhydrazine, cat. H_2SO_4, 3Å molecular sieves, dry EtOH; then carbobenzoxy chloride, 1*N* NaOH, CH_2Cl_2. *ii*, $CuCl_2$, aq. THF, phosphate buffer. *iii*, TBSOTf, Et_3N, dry CH_2Cl_2. *iv*, NBS, dry THF. *v*, DBU, dry DMF. *vi*, $NaBH_4$, MeOH. *vii*, TBDPSCl, Et_3N, DMAP, dry CH_2Cl_2. *viii*, BH_3-Me_2S, dry THF, then 3*N* NaOH, 31% aq. H_2O_2; then *n*-Bu_4NF, THF. *ix*, *n*-Bu_4NF, THF. *x*, $Pb(OAc)_4$, dry CH_2Cl_2. *xi*, 10% Pd-C, H_2, MeOH. *xii*, aq. formaline, cat. AcOH, $NaCNBH_3$.

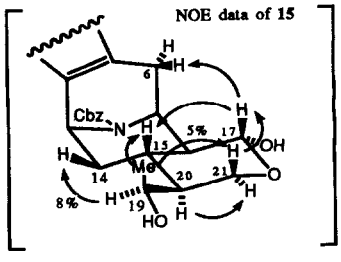


Fig. 3

Experimental

IR spectra were measured with a Hitachi 260 spectrophotometer, and UV spectra were measured in ethanol with a Hitachi U3400 spectrophotometer. ^{1}H NMR spectra were recorded on JEOL JNM FX-270, JNM GX-270 (270MHz), JEOL GSX500 and JNM-A500 (500MHz) spectrometers with tetramethylsilane as an internal standard. ^{13}C NMR spectra were measured with JEOL GSX400 (100.4MHz) and JNM GX-270 (67.8MHz) spectrometers with tetramethylsilane as an internal standard. Mass spectra were taken with Hitachi RMU-6E and RMU-7M, and JEOL JMS-HX110A spectrometers. Optical rotations were measured on a JASCO DIP-140 polarimeter. 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-HS-221-05 (for medium pressure column chromatography; MPLC)].

Isolation of 19(*S*)-hydroxy-N_b-methylraumacline (4) from cells and medium.

Rauwolfia serpentina cell suspension cultures were cultivated in the presence of ajmaline (1 g/l medium) as recently described.[3] After filtration suspension, cells were freeze dried, which (15 g) were extracted with MeOH and analyzed by TLC for CAS (ceric ammonium sulphate) reaction using silica gel plates and solvent system ($CHCl_3$/MeOH/ammonia=75:25:0.1). The band at R_f 0.28 (CAS greenish grey) was eluted, evaporated and rechromatographed yielding 0.5 mg of 19(*S*)-hydroxy-N_b-methylraumacline. Isolation from the nutrition medium was performed as follows. After extensive extraction of 6 l medium with CH_2Cl_2 at $_p$H 9.5, the organic layer was evaporated and the residue (1.3 g) was purified by flash chromatography with the above mentioned solvent system. Fractions showing 19-hydroxy-N_b-methylraumacline as one of the major alkaloids on TLC, were combined and dried, yielding 125 mg of the pre-purified alkaloid mixture. Final purification was achieved by preparative TLC on 0.5 mm plates with $CHCl_3$/MeOH/ammonia=8:2:0.02 (Rf=0.2) and then with ethyl acetate/MeOH/water/ammonia=7:2:1:0.02 (R_f=0.24), resulting in 10 mg of 19(*S*)-hydroxy-N_b-methylraumacline as an amorphous powder. $[\alpha]_D^{25}$ -86.7° (*c*=0.18, $CHCl_3$) (Found: M^+, 356.2099. $C_{21}H_{28}N_2O_3$ requires *M*, 356.2098); λ_{max} (EtOH) 290, 282, 223 nm; δ_H(500 MHz, acetone-D_6) 7.44 (1H, dd, *J* 7.5, 1.0, 9-H), 7.33 (1H, dd, *J* 8.1, 1.0, 12-H), 7.10 (1H, ddd, *J* 8.1, 7.1, 1.0, 11-H), 7.01 (1H, ddd, *J* 7.5, 7.1, 1.0, 10-H), 5.36 (1H, br-s, OH), 4.55 (1H, d, *J* 8.8, 17-H), 4.06 (1H, br-t, 3-H), 3.93 (1H, dd, *J* 11.3, 4.2, 21α-H), 3.82 (1H, m, 19-H), 3.68 (3H, s, N_a-CH_3), 3.47 (1H, br-d, *J* 4.0, OH), 3.43 (1H, dd, *J* 6.7, 4.5, 5-H), 3.20 (1H, dd, *J* 11.3, 11.3, 21β-H), 2.97 (1H, dd, *J* 16.8, 6.7, 6α-H), 2.61 (1H, d, *J* 16.8, 6β-H), 2.37 (3H, s, N_b-CH_3), 2.00 (1H, ddd, *J* 12.0, 4.2, 3.0, 14β-H), 1.74 (1H, m, 16-H), 1.71 (1H, m, 14α-H), 1.59 (1H, dddd, *J* 11.4, 11.3, 4.4, 4.2, 20-H), 1.24 (1H, qd, *J* 11.4, 4.2, 15-H), 0.68 (3H, d, *J* 6.6, 18-H); δ_C(125 MHz, acetone-D_6) 138.13 (C13), 135.34 (C2), 127.50 (C8), 121.27 (C11), 119.33 (C10), 118.57 (C9), 109.67 (C12), 107.09 (C7), 97.87 (C17), 66.00 (C19), 65.52 (C21), 53.72 (C5), 53.57 (C3), 51.73 (C16), 47.80 (C20), 41.74 (N_b-Me), 34.45 (C14), 32.38 (C15), 29.25 (N_a-Me), 18.25 (C18), 17.29 (C6); *m/z* 356 (M^+, 89%), 279 (10), 197 (100), 183 (28), 170 (17), 86 (13).

Preparation of the carbamate (5) from ajmaline (1)

A mixture of ajmaline (1) (500 mg, 1.532 mmol), *N, N*-dimethylhydrazine (0.58 ml, 7.634 mmol), a catalytic amount of H_2SO_4 (5 drops), molecular sieves 3 Å and ethanol (10 ml) was heated under reflux for 3 h. The filtrate obtained upon the filtration of molecular sieves was concentrated under reduced pressure and then basified with aq. NH_4OH solution. The aqueous layer was extracted with chloroform and the organic layer was washed with water, dried ($MgSO_4$), and evaporated. The residue was dissolved in 1*N*-NaOH/CH_2Cl_2=1:4 (12.5 ml) and carbobenzoxy chloride (0.25 ml, 1.751 mmol) was added to the mixture at 0 °C. The reaction mixture was stirred at room temperature for 30 min. The organic layer was separated and the aqueous layer was extracted with chloroform. The combined organic layers were washed with water, dried ($MgSO_4$) and evaporated. The residue was separated by MPLC with ethyl acetate-hexane (2:1) to give the carbamate (5) (600 mg, 80%) as an amorphous powder, λ_{max} (EtOH) 291, 246 and 206 nm; ν_{max} ($CHCl_3$) 3400, 2950 and 1690 cm^{-1}; δ_H(270MHz; $CDCl_3$) 6.43 and 6.42[8] (1H, each d, *J* 7.4 , 21-H), 5.23 and 5.09 (1H, each d, *J* 12.8, CO_2CH_2Ph), 5.21 and 5.08 (1H, each d, *J* 12.5, CO_2CH_2Ph), 2.77, 2.74, 2.73 and 2.62 (9H, each s, 3xNMe); *m/z* 502 (M^+, 50%), 358 (16), 144 (59) and 91 (100).

Hydrolysis of the hydrazone (5)

Copper (II) chloride was added portionwise to a solution of **5** (5.340 g, 10.62 mmol) in a mixture of THF (190 ml), H_2O (25 ml) and phosphate buffer (0.05 N, pH 7, 75 ml) at room temperature in the following manner: 0 min-3.588 g (26.69 mmol); 15.5 h-1.686 g (12.54 mmol); 63.5 h-428 mg (3.18 mmol). After the final additional of $CuCl_2$ the mixture was stirred at room temperature for 24 h. After concentration of ethanol the reaction mixture was diluted with ice-water and basified with aq. NH_4OH solution. The whole mixture was extracted with chloroform and the extract was washed with water, dried ($MgSO_4$) and evaporated. The residue was purified by silica gel flash column chromatography with 30-50% ethyl acetate-hexane to give **6** (4.123 g, 84%) as an amorphous powder, (Found: M^+, 460.2365. $C_{28}H_{32}N_2O_4$ requires M, 460.2360); λ_{max} (EtOH) 291, 247 and 205 nm; ν_{max} ($CHCl_3$) 3600, 3450, 1715, 1680 and 1420 cm^{-1}; δ_H (270MHz; $CDCl_3$) 9.61 (d, *J* 3.4) and 9.54 (d, *J* 4.3) (1 H, 21-H), 2.76 and 2.64 (3 H, each s, NMe); *m/z* 460 (M^+, 59%), 272 (41), 173 (36), 144 (47) and 91 (100).

Preparation of the silyl enol ether (7)

tert-Butyldimethylsilyl trifluoromethanesulphonate (TBSOTf) (0.24 ml, 1.045 mmol) was added to a solution of **6** (160 mg, 0.347 mmol) and triethylamine (0.22 ml, 1.581 mmol) in dry dichloromethane (1 ml) and the mixture was stirred for 1.5 h. Further TBSOTf (40 ml, 0.174 mmol) was added to the reaction mixture at 0 °C and the mixture was stirred at the same temperature for 1 h. A cold 5% sodium carbonate solution was added to the mixture and the whole was extracted with chloroform. The residue was separated by silica gel flash column chromatography with 10% ethyl acetate-hexane to give **7** (170 mg, 71%) as an amorphous powder, λ_{max} (EtOH) 293, 248 and 210 nm; ν_{max} ($CHCl_3$) 2950, 1690, 1100 and 850 cm^{-1}; δ_H (270MHz; $CDCl_3$) 6.18 (1 H, s, 21-H), 0.98, 0.97, 0.92 and 0.90 (18 H, each s, $SiBu^t$x2), 0.14, 0.11, 0.09 and 0.06 (12 H, each s, $SiMe_2$x2); *m/z* 689 (M^+, 18%), 412 (20), 236 (13), 144 (13) and 91 (100).

Bromination of the silyl enol ether (7)

A solution of *N*-bromosuccinimide (NBS) (65 mg, 0.365 mmol) in dry THF (2.5 ml) was added dropwise to a solution of **7** (227 mg, 0.329 mmol) in dry THF (2.5 ml) at -22 °C and the mixture was stirred at the same temperature for 2h. Saturated aqueous ammonium chloride solution was added and the whole was extracted with chloroform. The organic layer was washed with water, dried ($MgSO_4$) and evaporated. The residue was purified by MPLC with ethyl acetate-hexane (1:4) to yield **8** (167 mg, 78%) as an amorphous powder, λ_{max} (EtOH) 291, 248 and 208 nm; ν_{max} ($CHCl_3$) 1720, 1690, 1090 and 840 cm^{-1}; δ_H (270MHz; $CDCl_3$) 9.38 (d, *J* 0.9) and 9.31 (d, *J* 0.6) (1 H, 21-H); *m/z* 654 (M^++2, 3%), 652 (M^+, 2), 386 (20), 144 (25) and 91 (100).

Preparation of α,β-unsaturated aldehydes from the bromide (8)

1,8-Diazabicyclo[5,4,0]undec-7-ene (DBU) (0.60 ml, 4.012 mmol) was added to a solution of **8** (2.170 g, 3.320 mmol) in dry DMF (44 ml) and the mixture was stirred at room temperature for 65 h. Saturated aqueous ammonium chloride solution was added and the whole was extracted with chloroform. The extract was washed with water, dried ($MgSO_4$) and evaporated. DMF was removed by Kugelrohr apparatus and the residue was purified by silica gel flash column chromatography with 15-20% ethyl acetate-hexane to afford *Z*-olefin (**9**) (1346 mg, 71%) and *E*-olefin (**10**) (224 mg, 21%). (**9**): amorphous powder; (Found, HRFABMS, in *m*-nitrobenzylalcohol, MH^+, 573.3140, $C_{34}H_{45}O_4N_2Si$ requires MH, 573.3148) λ_{max} (EtOH) 292, 237 and 205 nm; ν_{max} ($CHCl_3$) 1690, 1680, 1430, 1090 and 840 cm^{-1}; δ_H (400MHz; $CDCl_3$) 10.13 and 10.09 (1 H, each s, 21-H), 6.62 (1 H, q-like, 19-H), 1.97 and 1.90 (3 H, each d, *J* 7.5, 18-Me); *m/z* 572 (M^+, 35%), 384 (47), 288 (19), 157 (20), 144 (32), 91 (100) and 73 (29). (**10**): amorphous powder; λ_{max} (EtOH) 292, 249 (sh), 227 and 206 nm; ν_{max} ($CHCl_3$) 1690, 1433, 1090 and 840 cm^{-1}; δ_H (500MHz; $CDCl_3$) 9.30 and 9.29 (1 H, each s, 21-H), 6.50 and 6.46 (1 H, each q, *J* 7.5, 19-H), 2.00 and 1.91 (3 H, each d, *J* 7.4, 18-Me); *m/z* 572 (M^+, 31%), 384 (45), 288 (19), 157 (20), 144 (35), 91 (100) and 73 (30).

$NaBH_4$ reduction of Z-olefin (9)

Sodium borohydride (7.3 mg, 0.192 mmol) was added to a solution of **9** (101 mg, 0.176 mmol) in methanol (4 ml) and the mixture was stirred at room temperature for 40 min. Water was added to the reaction mixture and the whole was extracted with

chloroform. The organic layer was washed with water, dried ($MgSO_4$) and evaporated. The residue was separated by MPLC with ethyl acetate-hexane (2:1) to afford **11** (100 mg, 98%) as an amorphous powder, λ_{max} (EtOH) 292, 245 and 206 nm; ν_{max} ($CHCl_3$) 3600, 3400, 1690, 1430, 1090, 1070 and 840 cm^{-1}; δ_H (270MHz; $CDCl_3$) 5.51(q-like, *J* 7.0) and 5.46 (q-like) (1H, 19-H), 4.21 (1H, d, *J* 11.9, 21-H), 4.12 (1H, d, *J* 12.2, 21-H), 1.62 (d, *J* 6.7) and 1.57 (d, *J* 7.0) (3H, 18-Me); *m/z* 574 (M^+, 33%), 386 (38), 288 (27), 144 (26), 91 (100) and 73 (35).

Protection of the Primary alcohol (11)

tert-Butyldiphenylsilyl chloride (TBDPSCl) (0.1 ml, 0.385 mmol) was added to a solution of the alcohol (**11**) (99 mg, 0.172 mmol), triethylamine (0.15 ml, 1.076 mmol) and *p*-dimethylaminopyridine (2.6 mg, 0.021 mmol) in dry dichloromethane (3 ml) and the mixture was stirred for 7 h at room temperature. Further TBDPSCl (0.05 ml, 0.192 mmol) was added to the reaction mixture and the mixture was then stirred at the same temperature for 15 h. Cold aqueous 5% sodium carbonate was added to the mixture and the whole was extracted with chloroform. The extract was washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by MPLC with 10% ethyl acetate-hexane to give the silyl ether (**12**) (140 mg, 100%) as an amorphous powder, (Found, HRFABMS, in *m*-nitrobenzylalcohol, MH^+, 813.4471, $C_{50}H_{65}O_4N_2Si_2$ requires *MH*, 813.4483) λ_{max} (EtOH) 291, 248, 222 (sh) and 205 (sh) nm; ν_{max} ($CHCl_3$) 1690, 1430, 1115, 1090, 1075 and 840 cm^{-1}; δ_H (270MHz; $CDCl_3$) 5.36(1H, br q, *J* 6.6, 19-H), 1.25 and 1.19 (3H, each d, *J* 6.6, 18-Me), 1.03 (9H), 0.979 and 0.970 (9H) (each s, Si*t*Bux2), 0.14 (6H, s, SiMex2); *m/z* 812 (M^+, 37%), 476 (14), 287 (33), 199 (26), 144 (28), 91 (100) and 73 (38).

Hydroboration of the olefin (12)

Boran methylsulfide complex (10 *M* sol., 122 ml, 1.240 mmol) was added to a solution of the olefin (**12**) (200 mg, 0.246 mmol) in dry THF at 0°C. The reaction mixture was then stirred at room temperature for 2.5 h. 3*N* aqueous NaOH (1.6 ml) and aqueous 31% hydrogen peroxide (0.36 ml) were successively added to the reaction mixture at 0°C and then the mixture was heated at 80-90°C for 1 h. Water was added, and the whole was extracted with chloroform. The organic layer was washed with water, dried ($MgSO_4$) and evaporated. The residue was dissolved in THF (4 ml) and a solution of *n*-Bu_4NF (1.0 M sol. in THF, 0.25 ml) was added to the mixture at 0°C. The reaction mixture was stirred at room temperature for 1 h, diluted with water and then extracted with chloroform. The extract was washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by MPLC with 50% ethyl acetate-hexane to give the alcohol (**13**) (44 mg, 30%) and the diastereomer (5.5 mg, 4%). **13** (Found, HRFABMS, in *m*-nitrobenzylalcohol, MH^+, 593.3397, $C_{34}H_{49}O_5N_2Si$ requires *MH*, 593.3341): λ_{max} (EtOH) 292, 247 and 205 nm; ν_{max} ($CHCl_3$) 3410, 1690, 1435, 1090 and 840 cm^{-1}; δ_H (270MHz; $CDCl_3$) 1.21 (d, *J* 6.3) and 1.19 (d, *J* 6.6) (3H, 18-Me), 0.97 and 0.96 (9H, each s, Si*t*Bu), 0.118, 0.113 and 0.105 (6H, each s, SiMex2); *m/z* 592 (M^+, 36%), 404 (30), 340 (27), 287 (21), 144 (25), 91 (100) and 73 (28).

Preparation of the triol (14)

A mixture of the silyl ether (**13**) (62 mg, 0.104 mmol) and *n*-Bu_4NF (1.0 *M* sol in THF, 0.12 ml) in THF (2 ml) was stirred at room temperature for 40 h. Water was added to the reaction mixture and the whole was extracted with 5% MeOH-$CHCl_3$. The organic layer was washed with brine and dried ($MgSO_4$). Removal of the solvent gave a residue, which was purified by MPLC with 2% MeOH-$CHCl_3$ to give the triol (**14**) (48 mg, 96%) as an amorphous powder, λ_{max} (EtOH) 291, 248 and 205 nm; ν_{max} ($CHCl_3$) 3400, 1680, 1440 and 1110 cm^{-1}; δ_H (270MHz; $CDCl_3$) 4.40 (1H, m, 19-H), 1.18 (3H, d, *J* 6.3, 18-Me); *m/z* 478 (M^+, 34%), 226 (12), 173 (36), 144 (35) and 91 (100).

Lead tetraacetate oxidation of the indoline (14)

To a stirred solution of the indoline (**14**) (45 mg, 0.094 mmol) in dry CH_2Cl_2 (2 ml) was added $Pb(OAc)_4$ (53 mg, 0.108 mmol) at -70°C under nitrogen atmosphere. After 2.5 h the reaction mixture was diluted with $CHCl_3$ and washed with aqueous 1*N* sodium carbonate solution. The aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with brine, dried ($MgSO_4$) and evaporated. The residue was purified by MPLC with ethyl acetate to afford the indole (**15**) (38.5 mg, 88%) as an

amorphous powder. (Found M^+, 476.2288, $C_{28}H_{32}O_5N_2$ requires M, 476.2311), λ_{max} (EtOH) 293 (sh), 284 and 229 nm; ν_{max} ($CHCl_3$) 3370, 1680, 1430, 1130 and 1090 cm^{-1}; δ_H (270MHz; $CDCl_3$) 5.58 and 5.48 (1H, each br s, 3-H), 4.54 (d, J 8.9) 4.51 (d, J 9.6) (1H, 17-H), 3.79 (1H, m, 19-H), 0.79 and 0.78 (3H, each d, J 6.6, 18-Me); *m/z* 476 (M^+, 25%), 458 (25), 432 (28), 273 (22), 183 (45), 170 (32) and 91 (100).

Preparation of 19(*S*)-Hydroxy-N_b-methylraumacline (4).

A mixture of the carbamate (**15**) (250 mg, 0.524 mmol) and 10% Pd-C (295 mg) in MeOH (15 ml) was stirred vigorously under hydrogen at 1 atm for 4.5 h. The catalyst was filtered off and the filtrate was concentrated to give the crude amine (**16**) (210 mg), which was directly subject-ed to the next reaction. To a stirred mixture of the amine (**16**) and acetic acid (4.5 ml) in 37% aqueous formaline (60 ml) was added $NaBH_3CN$ (650 mg, 10.34 mmol) at 0°C. The reaction mixture was stirred at room temperature for 3 h. The mixture was diluted with cold water, neutralized with saturated aqueous sodium bicarbonate, and then extracted with 5% MeOH-$CHCl_3$. The extract was washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by flash column chromatography with 20-30% MeOH-$CHCl_3$ to give **4** (135 mg, 72% from **15**) as an amorphous powder, $[\alpha]_D^{25}$ -90.5° (c=0.2, $CHCl_3$); (HRFABMS in *m*-nitrobenzylalcohol, Found: MH^+, 357.2187. $C_{21}H_{29}N_2O_3$ requires MH, 357.2179). The synthetic compound was identical with authentic sample on comparison of their chromatographic behavior and spectral data (MS, ^{1}H- and ^{13}C-NMR).

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8 Ajmaline derivatives possessing a carbamate function are often shown by NMR spectra to occur as a mixture of rotation isomers.