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Quinolizidines. XIX.[1] Synthesis of (−)-9-Demethyltubulosine

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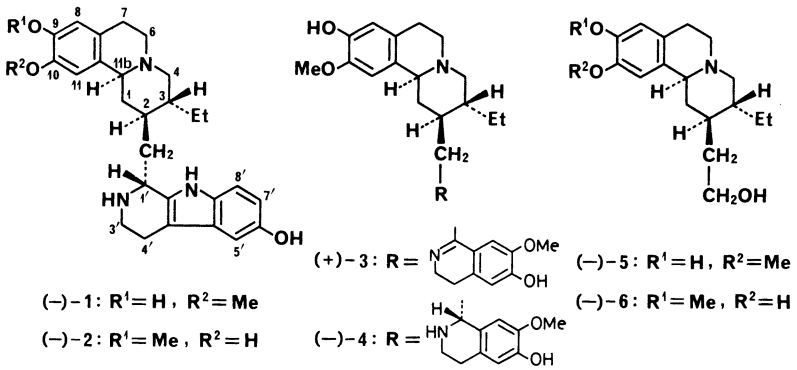
The chiral synthesis of (−)-9-demethyltubulosine [(−)-**1**] has been achieved for the first time *via* a "cincholoipon-incorporating route," which started from cincholoipon ethyl ester [(+)-**7**] and passed through the intermediates (−)-**8**, (−)-**9**, (+)-**10**, and (+)-**11**. As a result, the absolute configuration of the *Alangium vitiense* alkaloid 9-demethyltubulosine has been unequivocally established to be that represented by formula (−)-**1**.

Keywords——*Alangium vitiense* alkaloid; demethyltubulosine; chiral synthesis; demethylisotubulosine; diethyl phosphorocyanidate amide formation; Bischler–Napieralski cyclization; carbon–nitrogen double-bond catalytic reduction; benzyl ether hydrogenolysis; CD epimer differentiation

(−)-9-Demethyltubulosine [(−)-**1**][2] is an antitumor alkaloid isolated by Husson's group[3] from the trunk bark of *Alangium vitiense* (A. GRAY) BAILLON (Alangiaceae), a plant collected in Vanuatu, New Hebrides.[4] They have reported that this alkaloid increased the survival time of mice infected with leukemia L1210 or P388.[3b] Its chemical structure and relative stereochemistry were determined[4] by analysis of the spectral data and conclusively by a direct comparison with synthetic (±)-**1**.[5] As regards the absolute stereochemistry, that represented by formula (−)-**1** was assigned[4] on the basis of the similarity in circular dichroism (CD) spectrum to the known *A. lamarckii* alkaloid (−)-10-demethyltubulosine [(−)-**2**].[6] This paper details the results of our efforts toward a chiral synthesis of the candidate structure (−)-**1**, which have confirmed the correctness of the above stereochemical assignment.[7]

Our previous work has shown that unified chiral syntheses of all four groups of benzo[*a*]quinolizidine-type *A. lamarckii* alkaloids[8] are possible through routes adopting the "cincholoipon-incorporating strategy."[9] In view of the fact that the target structure (−)-**1**



falls within the 9-hydroxy-10-methoxybenzo[*a*]quinolizidine-type group, we tried to extend the scope of this synthetic strategy to include the synthesis of (−)-**1**. The starting point in the synthetic scheme was the known tricyclic amino acid (−)-**8**, a common key intermediate synthon utilized for our recent unified syntheses of 9-demethylpsychotrine [(+)-**3**],[10] 9-demethylcephaeline [(−)-**4**],[11] and 9-demethylprotoemetinol [(−)-**5**].[12] The tricycle (−)-**8** was available from cincholoipon ethyl ester [(+)-**7**][13] by the previously reported 11-step synthesis,[10] and the reaction sequence thereafter paralleled that employed by us[5] for the racemic synthesis of **1** from (±)-**8**.

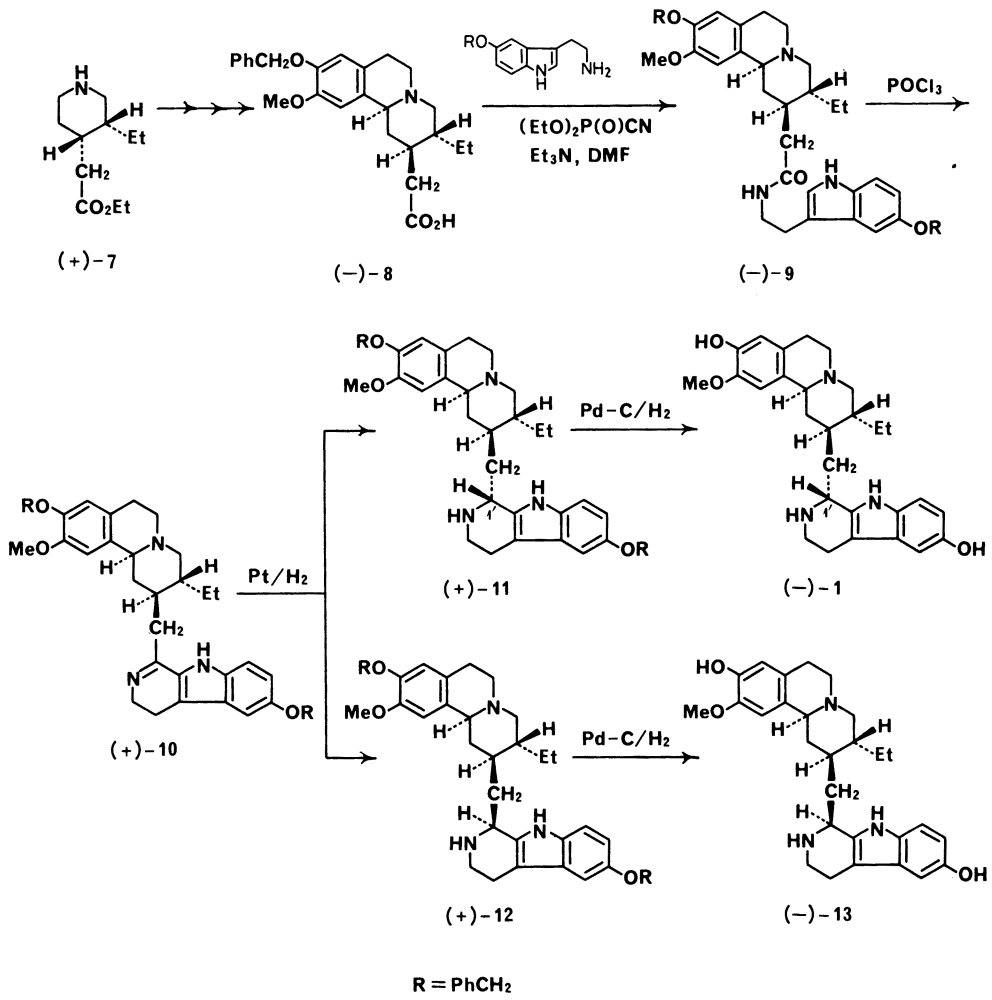


Chart 1

Thus, (−)-**8** and 5-benzyloxytryptamine[14] were coupled in *N*,*N*-dimethylformamide (DMF) by the diethyl phosphorocyanidate method,[15] giving the amide (−)-**9** in 86% yield. Bischler–Napieralski cyclization of (−)-**9** with $POCl_3$ in boiling toluene furnished the dihydro-β-carboline (+)-**10** (63% yield), which was then submitted to catalytic hydrogenation in dioxane over Adams catalyst. The resulting hydrogenation products were separated by column chromatography to give *O*,*O*-dibenzyl-9-demethyltubulosine [(+)-**11**] and its 1′-epimer [(+)-**12**] in 31% and 57% yields, respectively. On hydrogenolysis using hydrogen and

Pd–C catalyst, (+)-**11** produced the target molecule (−)-**1** in 90% yield. The epimeric base (+)-**12** was debenzylated similarly, providing 9-demethylisotubulosine [(−)-**13**] in 86% yield. The stereochemistry of the newly formed asymmetric center at C-1′ of (+)-**11**, (+)-**12**, (−)-**1**, and (−)-**13** was confirmed by the identity of their solution infrared (IR) (in $CHCl_3$)[16] and nuclear magnetic resonance (NMR) spectra and thin-layer chromatographic (TLC) mobility with those of the corresponding racemic modifications[5] of established stereochemistry. The synthetic (−)-**1** proved to be identical with a natural sample of the *A. vitiense* alkaloid 9-demethyltubulosine by a direct comparison of the TLC mobility and ultraviolet (UV), IR, and ^{1}H- and ^{13}C-NMR spectra, and especially of the specific rotation and CD spectrum.

In summary, the results of the above chiral synthesis have defined the absolute configurations of the four asymmetric centers in the $C_{28}H_{35}N_3O_3$ *A. vitiense* alkaloid as shown in formula (−)-**1**. Interestingly, the 10-demethyl isomer (−)-**2**[6] and 10-demethylprotoemetinol [(−)-**6**][12,17] as well as the 9-demethylated congeners, such as 9-demethylpsychotrine [(+)-**3**][6b,10] and 9-demethylprotoemetinol [(−)-**5**],[12,17,18] have been found in *Alangium lamarckii* THWAITES, another species of the same genus. It is hoped that the newly established synthetic route to (−)-**1** will facilitate the supply of a sufficient amount of this alkaloid for further assessment of its oncostatic activity and for other biological tests.

Experimental

General Notes——All melting points were taken on a Yamato MP-1 capillary melting point apparatus and are corrected. See ref. 1 for details of instrumentation and measurements. Microanalyses were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, m = multiplet, s = singlet, sh = shoulder, t = triplet.

(2*R*,3*R*,11b*S*)-9-Benzyloxy-*N*-[2-(5-benzyloxy-1*H*-indol-3-yl)ethyl]-3-ethyl-1,3,4,6,7,11b-hexahydro-10-methoxy-2*H*-benzo[*a*]quinolizine-2-acetamide [(−)-9]——A solution of (−)-**8**[10] (311 mg, 0.76 mmol) and 5-benzyloxytryptamine[14] (304 mg, 1.14 mmol) in $HCONMe_2$ (5 ml) was stirred under ice-cooling, and diethyl phosphorocyanidate[19] (248 mg, 1.52 mmol) and Et_3N (154 mg, 1.52 mmol) were added in that order. The mixture was stirred at room temperature for 6 h and extracted, after addition of H_2O (15 ml), with $CHCl_3$. The $CHCl_3$ extracts were washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave a brown glass. The glass was crystallized from AcOEt to give (−)-**9** (431 mg, 86%) as colorless needles. Recrystallization from AcOEt furnished an analytical sample, mp 168—168.5 °C; $[\alpha]_D^{19}$ −8.0° (*c* = 0.50, EtOH); MS *m/z*: 657 (M^+); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490 and 3460 (NH's), 2810 and 2750 (*trans*-quinolizidine ring),[20] 1658 (amide CO); ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 6.6 Hz, $CCH_2\underline{Me}$), 3.78 (3H, s, OMe), 5.09 (4H, s, two $OC\underline{H}_2Ph$'s), 5.54 (1H, t, *J* = 5.5 Hz, CONH), 6.61 (1H, s, $H_{(8)}$ or $H_{(11)}$), 6.70 (1H, s, $H_{(11)}$ or $H_{(8)}$), 6.93 (1H, dd, *J* = 9.3, 2.2 Hz, $H_{(6')}$), 6.97 (1H, d, *J* = 2.4 Hz, $H_{(2')}$), 7.12 (1H, d, *J* = 2.2 Hz, $H_{(4')}$), 7.22 (1H, d, *J* = 9.3 Hz, $H_{(7')}$), 7.2—7.5 (10H, m, two $OCH_2\underline{Ph}$'s), 8.07 (1H, br, indole NH).[21] *Anal.* Calcd for $C_{42}H_{47}N_3O_4$: C, 76.68; H, 7.20; N, 6.39. Found: C, 76.62; H, 7.28; N, 6.39.

(2*R*,3*R*,11b*S*)-9-Benzyloxy-2-[(6-benzyloxy-4,9-dihydro-3*H*-pyrido[3,4-*b*]indol-1-yl)methyl]-3-ethyl-1,3,4,6,7,11b-hexahydro-10-methoxy-2*H*-benzo[*a*]quinolizine [(+)-10]——A solution of (−)-**9** (1.45 g, 2.2 mmol) and $POCl_3$ (3.37 g, 22 mmol) in dry toluene (60 ml) was heated under reflux in an atmosphere of nitrogen for 2.5 h. The reaction mixture was concentrated *in vacuo*, and 5% aqueous KOH (50 ml) and CH_2Cl_2 (50 ml) were added to the oily residue under ice-cooling. The CH_2Cl_2 layer was separated from the aqueous layer, which was further extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave a brown glass. Purification of the glass by column chromatography [alumina, CH_2Cl_2–EtOH (50 : 1, v/v)] afforded (+)-**10** (892 mg, 63%) as a pale yellow glass, $[\alpha]_D^{27}$ +34.6° (*c* = 1.00, EtOH); MS *m/z*: 639 (M^+); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490 (NH), 2760 (*trans*-quinolizidine ring)[20]; ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, *J* = 6.8 Hz, $CCH_2\underline{Me}$), 3.53 (3H, s, OMe), 5.04 and 5.10 (2H each, s, two $OC\underline{H}_2Ph$'s), 6.41 (1H, s, $H_{(8)}$ or $H_{(11)}$), 6.55 (1H, s, $H_{(11)}$ or $H_{(8)}$), 6.9—7.5 (13H, m, $H_{(5')}$, $H_{(7')}$, $H_{(8')}$, and two $OCH_2\underline{Ph}$'s), 8.34 (1H, br, NH).[22]

[2*S*-[2α(*S),3β,11bβ]]- and [2*S*-[2α(*R**),3β,11bβ]]-9-Benzyloxy-2-[(6-benzyloxy-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)methyl]-3-ethyl-1,3,4,6,7,11b-hexahydro-10-methoxy-2*H*-benzo[*a*]quinolizines [(+)-11 and (+)-12]**——A solution of (+)-**10** (608 mg, 0.95 mmol) in dioxane (15 ml) was hydrogenated over Adams catalyst (100 mg) at atmospheric pressure and 29 °C for 1.5 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to leave an orange oil, which was dissolved in $CHCl_3$ (50 ml). The $CHCl_3$ solution was washed successively with 5% aqueous NaOH and saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resulting pale orange glass was then chromatographed on a silica gel column using $CHCl_3$–EtOH (10 : 1, v/v) as the eluent. Earlier fractions gave *O*,*O*-dibenzyl-9-demethyltubulosine [(+)-**11**] (191 mg, 31%) as a pale

yellowish glass, $[\alpha]_D^{24}+7.6$ ($c=1.00$, EtOH); MS m/z: 641 (M^+). The IR ($CHCl_3$) and ^{1}H-NMR ($CDCl_3$) spectra of this sample were identical with those of authentic (±)-**11**.[5]

Later fractions in the above chromatography provided the 1′α-H isomer (+)-**12** (346 mg, 57%) as a pale yellowish glass, $[\alpha]_D^{24}+6.8$ ($c=1.00$, EtOH); MS m/z: 641 (M^+). The IR ($CHCl_3$) and ^{1}H-NMR ($CDCl_3$) spectra of this specimen were superimposable on those of authentic (±)-**12**.[5]

[2*S*-[2α(*S),3β,11bβ]]-3-Ethyl-1,3,4,6,7,11b-hexahydro-9-hydroxy-2-[(6-hydroxy-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)methyl]-10-methoxy-2*H*-benzo[*a*]quinolizine [(−)-9-Demethyltubulosine] [(−)-1]**——A solution of (+)-**11** (205 mg, 0.32 mmol) in MeOH–AcOH (1 : 1, v/v) (15 ml) was hydrogenated over 10% Pd–C (200 mg) at atmospheric pressure and 24 °C for 3 h. Removal of the catalyst by filtration and evaporation of the filtrate under reduced pressure left a pale yellowish oil, which was triturated with H_2O (5 ml). The resulting aqueous mixture was filtered, and the filtrate was made alkaline with 10% aqueous Na_2CO_3. The precipitate that resulted was filtered off, washed with H_2O, and dried to give (−)-**1** (132 mg, 90%) as a pale yellowish solid. Purification of the solid by column chromatography [alumina, $CHCl_3$–MeOH (10 : 1, v/v)] and trituration of the resulting yellow glass with ether produced a slightly yellowish powder, mp 203—205 °C; $[\alpha]_D^{25}-81.0$ ($c=1.00$, pyridine); MS m/z: 461 (M^+); CD ($c=8.23\times10^{-5}$ M, EtOH) $[\theta]^{22}$ (nm): 0 (318), +300 (314) (pos. max.), 0 (310), −2920 (295) (neg. max.), −2190 (288) (pos. max.), −3280 (277) (neg. max.), −120 (250) (pos. max.). The IR (Nujol), UV (MeOH, 0.1 N aqueous NaOH, 0.1 N aqueous HCl), ^{1}H-NMR (Me_2SO-d_6), and ^{13}C-NMR (Me_2SO-d_6) spectra and TLC mobility of this sample were identical with those (excepting the solid-state IR spectrum) of authentic (±)-**1**[5] as well as those of the $C_{28}H_{35}N_3O_3$ alkaloid [lit.[3,4] mp 200 °C; lit.[3,4] $[\alpha]_D^{20}-40$ ($c=1$, pyridine); CD ($c=8.40\times10^{-5}$ M, EtOH) $[\theta]^{18}$ (nm): 0 (318), +240 (314) (pos. max.), 0 (310), −2740 (294) (neg. max.), −2080 (288) (pos. max.), −2800 (276) (neg. max.), 0 (250) (pos. max.)[23]] isolated from *Alangium vitiense*.[3,4]

[2*S*-[2α(*R),3β,11bβ]]-3-Ethyl-1,3,4,6,7,11b-hexahydro-9-hydroxy-2-[(6-hydroxy-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)methyl]-10-methoxy-2*H*-benzo[*a*]quinolizine [(−)-9-Demethylisotubulosine] [(−)-13]**——Debenzylation of (+)-**12** and work-up of the reaction mixture were performed as described above for (−)-**1**, giving (−)-**13** (86% yield) as a slightly yellowish powder, mp 200—202 °C; $[\alpha]_D^{25}-98.8$ ($c=1.00$, pyridine); MS m/z: 461 (M^+); CD ($c=5.51\times10^{-5}$ M, EtOH) $[\theta]^{22}$ (nm): 0 (325), −4720 (310) (neg. max.), −3630 (300) (sh), 0 (294), +8890 (278) (pos. max.), +1270 (248) (neg. max.), +3630 (242) (pos. max.), 0 (238). The UV (MeOH, 0.1 N aqueous NaOH, 0.1 N aqueous HCl) and ^{1}H-NMR (Me_2SO-d_6) spectra and TLC mobility of this sample were identical with those of authentic (±)-**13**.[5]

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