Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 13.40 (q, Me C-2), 18.37 and 20.76 (q, Me C-6), 30.15 and 30.33 (t, C-3), 42.35 and 43.28 (d, C-2), 65.94 (d, C-6), 74.78 (d, Py C-2), 124.77 (d, C-4), 151.66 and 151.81 (d, C-5), 195.80 (s, Py C-3), 203.90 (s, C-1).

[*R*-[*R,*R***S**]]-3,6-Dihydro-α-methyl-3-oxo-2*H*-pyran-2-propanal (16)**: 66.5% (hexane–ethyl acetate–dichloromethane (3:2:5)); $[\alpha]^{20}_D$ = +14° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.17 (d, 1.5 H, *J* = 7.07 Hz, Me C-2), 1.18 (d, 1.5 H, *J* = 7.14 Hz, Me C-2), 1.9 (ddd, 0.5 H, *J* = 3.79, 8.72 and 14.41 Hz, H-3 (*R*)), 2–2.2 (m, 1 H, H-3 (*S*)), 2.4 (ddd, 0.5 H, *J* = 3.79, 8.72, and 14.41 Hz, H-3 (*R*)), 2.6 (m, 1 H, H-2), 4.1 (m, 1 H, Py H-2), 4.4 (m, 2 H, H-6), 6.15 (dd, 1 H, *J* = 1.98, 2.09, and 10.24 Hz, H-4), 7.1 (dd, 1 H, *J* = 2.21, 2.92, and 10.24 Hz, H-5), 9.62 (d, 0.5 H, *J* = 2.21 Hz, H-1), 9.64 (0.5 H, *J* = 1.9 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 13.73 and 20.97 (q, Me C-2), 30.61 (t, C-3), 42.44 and 43.01 (d, C-2), 63.90 and 64.23 (t, C-6), 78.15 and 78.61 (d, Py C-2), 126.49 (d, C-4), 148 (d, C-5), 195.90 (s, Py C-3), 204.42 (d, C-1).

[*S*-[*S,*R***S**]]-3,6-Dihydro-α-methyl-3-oxo-2*H*-pyran-2-propanal (17)**: 70% (hexane–ethyl acetate–dichloromethane (3:2:5)); $[\alpha]^{20}_D$ = −10 (*c* 0.5, $CHCl_3$); ^1H NMR δ 1.17 (d, 0.5 H, *J* = 7.06 Hz, Me C-2), 1.18 d, 0.5 H, *J* = 7.12 Hz, Me C-2), 1.9 (ddd, 0.5 H, *J* = 3.7, 8.51, and 14.80 Hz, H-3 (*S*)), 2–2.2 (m, 1 H, H-3 (*R*)), 2.4 (ddd, 0.5 H, *J* = 3.7, 8.51, and 14.80 Hz, H-3 (*S*)), 2.6 (m, 1 H, H-2), 4.1 (m, 1 H, Py H-2), 4.4 (m, 2 H, H-6), 6.15 (dd, 1 H, *J* = 1.85, 2.2, and 10.36 Hz, H-4), 7.1 (dd, 1 H, *J* = 1.87, 2.62, and 10.36 Hz, H-5), 9.62 (d, 0.5 H, *J* = 2.29 Hz, H-1), 9.64 (0.5 H, *J* = 1.87 Hz, H-1); ^{13}C NMR δ 13.70 and 20.94 (q, Me C-2), 30.59 (t, C-3), 42.42 and 43.37 (d, C-2), 63.98 and 64.20 (t, C-6), 78.15 and 78.59 (d, Py C-2), 126.44 (d, C-4), 147.98 (d, C-5), 195.59 (s, Py C-3), 204.28 (d, C-1).

[*R*-[*R,*R***S**]]-1,1-Bis(acetyloxy)-1,2-dideuterio-3,6-dihydro-α-methyl-3-oxo-2*H*-pyran-2-propanal (23)**: 70% (hexane–ethyl acetate–dichloromethane (3:2:5)); $[\alpha]^{20}_D$ = +45° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.01 (s, 1.62 H, Me C-2 (*R*)), 1.02 (s, 1.38 H, Me C-2 (*S*)), 1.6 (dd, 0.54 H, *J* = 8.85 and 14.8 Hz, H-3(*R*)), 1.87 (m, 0.92 H, H-3 (*S*)), 2.15 (two s, 6 H, CH_3CO), 2.18 (ddd, 0.54 H, *J* = 4.29 and 14.8 Hz, H-3 (*R*)), 4.05 (m, 0.54 H, Py H-2 (*R*)), 4.3 (m, 0.46 H, Py H-2 (*S*)), 4.45 (m, 2 H, H-6), 6.17 (ddd, 1 H, *J* = 2.86, 3.8, and 10.49 Hz, H-4), 7.1 (ddd, 1 H, *J* = 2.81, 3.81, and 10.49 Hz, H-5).

[2*S*-[2α(*R*S**),5α,6β]]-5-(Acetyloxy)-α,6-dimethyl-5,6-dihydro-2*H*-pyran-2-propanal (25)**: $[\alpha]^{20}_D$ = −77° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.14 (s, 2 H, Me C-2 (*S*)), 1.16 (s, 1 H, Me C-2 (*R*)), 1.21 (d, 1 H, *J* = 6.7 Hz, Me C-6 (*R*)), 1.38 (d, 2 H, *J* = 6.7 Hz, Me C-6 (*S*)), 1.42 (dd, 0.66 H, *J* = 3.64 and 14.58 Hz, H-3 (*S*)), 1.78 (dd, 0.33 H, *J* = 9.11 and 14.58 Hz, H-3 (*R*)), 1.94 (dd, 0.33 H, *J* = 3.95 and 14.58 Hz, H-3 (*R*)), 2.1 (s, 3 H, CH_3CO), 2.15 (dd, 0.66 H, *J* = 10.32 and 14.58 Hz, H-3 (*S*)), 3.85 (dq, 1 H, *J* = 5.6 and 7 Hz, H-6), 4.25 (m, 1 H, Py H-2), 4.86 (m, 1 H, H-5), 5.74–5.92 (m, 2 H, Py H-3 and H-4).

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Ircinals A and B from the Okinawan Marine Sponge *Ircinia* sp.: Plausible Biogenetic Precursors of Manzamine Alkaloids

Kazuhiko Kondo,[1a] Hideyuki Shigemori,[1a] Yumiko Kikuchi,[1a] Masami Ishibashi,[1a] Takuma Sasaki,[1b] and Jun'ichi Kobayashi*[,1a]

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan, and Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan

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The manzamines, which have been isolated from marine sponges,[2-4] comprise a new group of β-carboline alkaloids having intricate polycyclic systems. The provenance of the nitrogen-containing ring systems embraced in the manzamine alkaloids has been problematical since there appears to be no obvious biogenetic path. During our studies on bioactive substances from Okinawan marine organisms,[5] we have isolated two novel alkaloids, ircinals A (1) and B (2), which might be plausible biogenetic precursors of the manzamine alkaloids, from the Okinawan marine sponge *Ircinia* sp. Here, we describe the isolation and structure elucidation of 1 and 2 as well as two new manzamine congeners, manzamines H (3) and J (4), from the same sponge. Ircinals A (1) and B (2) were converted into manzamines A (5) and J (4), respectively, through Pictet–Spengler cyclization with tryptamine followed by DDQ oxidation. Ircinals A (1) and B (2) and manzamines H (3) and J (4) exhibited cytotoxicity.

The sponge *Ircinia* sp. was collected off Kise, Okinawa, and kept frozen until processing. The methanol extract of the sponge was partitioned between ethyl acetate and water. The ethyl acetate soluble material was subjected

Table I. ^1H and ^{13}C NMR Chemical Shifts (ppm) of Ircinal A (1) and Protons to which Long-Range Correlations Were Observed in the HMBC Experiments

position	^{13}C	^1H	*J* (Hz)	HMBC (^1H)
1	193.3 d	9.45 s		H-11, H-24
10	142.6 s			H-1, H-23, H-24
11	157.6 d	6.75 s		H-13, H-24, H-26
12	70.2 s			H-13, H-26
13	38.9 t	1.61 m, 1.77 m		H-14, H-26
14	21.0 t	2.11 m, 2.24 m		H-15, H-16
15	127.9 d	5.56 m		H_2-14
16	132.5 d	5.50 dddd	10.7, 10.7, 10.7, 4.6	H-14
17	25.6 t	1.63 m, 2.43 m		H-15, H-16
18	26.7 t	1.25 m, 1.41 m		H-20
19	25.3 t	1.38 m, 1.71 m		H_2-20
20	53.5 t	2.39 m, 2.57 m		H-36
22	49.4 t	1.86 m, 2.77 m		H-20, H-23, H_2-36
23	31.6 t	1.22 m, 1.78 m		H-24
24	34.0 d	2.58 dd	12.2, 6.8	H-11, H-22, H-23, H-35, H-36
25	46.4 s			H-24, H-26, H-34, H-35, H-36
26	76.3 d	3.44 s		H-11, H-13, H-24, H-34, H-35, H_2-36
28	51.4 t	3.04 m, 3.38 m		H-26
29	29.8 t	1.73 m, 1.93 m		H-28, H-31
30	25.3 t	1.35 m, 1.89 m		H_2-28, H_2-31, H-32
31	28.2 t	2.14 m, 2.29 m		H-32, H-33
32	137.1 d	6.03 dddd	11.0, 7.1, 7.1, 1.5	H-30, H_2-31, H-33, H-34
33	127.7 d	5.26 ddd	10.5, 10.3, 1.2	H-34, H_2-35
34	55.4 d	4.36 br t	8.1	H-28, H-32, H-33, H-35
35	44.6 t	1.67 m, 1.86 m		H-24, H-26, H-33, H-34, H_2-36
36	69.2 t	2.29 d, 2.81 d	11.2	H-22, H-26, H_2-35

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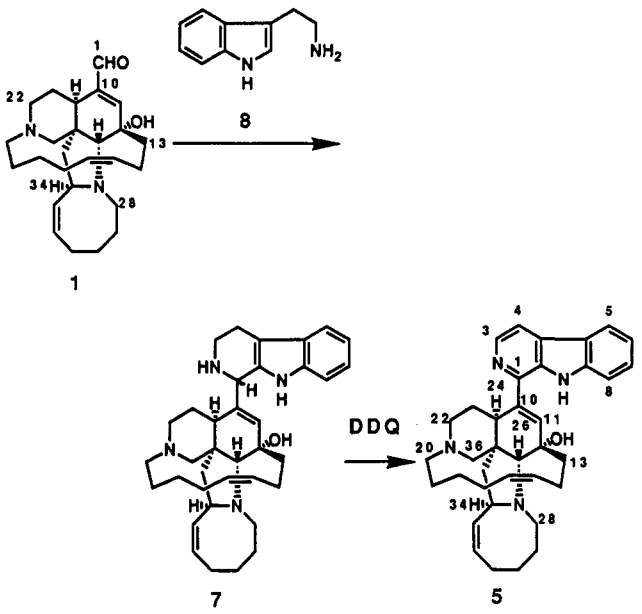
Scheme I



to silica gel chromatography (hexane/acetone (4:1), $CHCl_3$/MeOH (95:5), and hexane/acetone (9:1)) to afford ircinals A (1, 0.0057% wet weight of the sponge) and B (2, 0.0020%) and manzamines H (3, 0.0007%) and J (4, 0.0022%) together with the known β-carboline alkaloids, manzamines A[2a] (5, 0.071%), B[2b] (6, 0.012%), and D[6] (7, 0.0023%).

HREIMS of 1 suggested the molecular formula as $C_{26}H_{38}N_2O_2$ (m/z 410.2924, M^+, Δ −0.9 mmu). The IR spectrum indicated the presence of hydroxyl (3420 cm^{-1}) and unsaturated carbonyl (1680 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of 1 showed the presence of one aldehyde group (δ_H 9.45 and δ_C 193.3) and three (two di- and one trisubstituted) double bonds (δ_H 6.75, 6.03, 5.56, 5.50, and 5.26; δ_C 157.6, 142.6, 137.1, 132.5, 127.9, and 127.7). Since four of the nine unsaturations present in the molecule were thus accounted for, 1 was assumed to possess five rings which included two nitrogen atoms. The assignment of all protonated carbons was established by a heteronuclear single quantum coherence (HSQC)[7] experiment (Table I). Analyses of the ^1H–^1H COSY spectrum of 1 allowed assignment of all protons and revealed the proton connectivities for three partial structures, C-13 to C-20, C-22 to C-24, and C-28 to C-35. Connection of three quaternary carbons (C-10, C-12, and C-25) and two nitrogen atoms (N-21 and N-27) with the three partial structures or the aldehyde group was achieved by the ^1H–^{13}C long-range correlations observed in the HMBC[8] spectrum (Table I): C-10 was shown to be coupled to C-1, C-11, and C-24; C-12 to C-11, C-13, and C-26; C-25 to C-24, C-26, C-35, and C-36; N-21 to C-20, C-22, and C-36; N-27 to C-26, C-28, and C-34. Thus, ircinal A had to have the structure depicted by 1. Since the ^1H NMR spectrum of 1 resembled that of manzamine A (5) except for a β-carboline moiety, the structure of 1 was rigorously confirmed by the following chemical conversion. Pictet–Spengler cyclization of 1 with tryptamine in the presence of trifluoroacetic acid afforded 1,2,3,4-tetrahydromanzamine A (= manzamine D, 7), which was transformed into manzamine A (5) through DDQ oxidation (Scheme I). The UV, IR, ^1H NMR, and EIMS spectra and $[\alpha]_D$ value of manzamine A (5), prepared from 1, were identical with those of an authentic sample.[2a]

HREIMS data of 2 (m/z 412.3118, M^+, Δ 2.9 mmu) agreed with the molecular formula $C_{26}H_{40}N_2O_2$. The IR spectrum suggested the presence of hydroxyl (3400 cm^{-1}) and unsaturated carbonyl (1680 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of 2 were similar to those of 1 except that the methine proton signal (δ 4.36; H-34) of 1 was not observed in the ^1H NMR spectrum of 2. The molecular ion of 2 at m/z 412 was two mass units larger than that of 1. These results suggested that the structure of ircinal B was 2 where the C–N bond between C-34 and N-27 in 1 was disconnected. The structure of 2 was further established by chemical correlation with manzamine B (6).[2b] Pictet–Spengler cyclization of 2 with tryptamine in the presence of trifluoroacetic acid afforded 1,2,3,4-tetrahydro-β-carboline 3, which was transformed into the corresponding β-carboline (4) through DDQ oxidation. Treatment of manzamine B (6) with NaH also gave compound 4 (Scheme II). The UV, IR, ^1H NMR, and EIMS spectra and $[\alpha]_D$ values of the two compounds (4) prepared from 2 and 6 were identical.

Manzamine H (3) was obtained as a colorless solid. HREIMS data of 3 (m/z 554.3980, M^+, Δ −0.5 mmu) coincided with the molecular formula $C_{36}H_{50}N_4O$. The UV absorptions (225, 277, 282, and 290 nm) indicated the presence of an indole chromophore. The IR spectrum suggested the presence of NH/OH (3400 and 3300 cm^{-1}). The ^1H and ^{13}C NMR spectra of 3 were similar to those of manzamine B (6). The aromatic proton signals (δ 8.26 and 7.84) of 6 were not observed in the ^1H NMR spectrum of 3, and this suggested 3 was a 1,2,3,4-tetrahydro-β-carboline. The ^1H and ^{13}C NMR spectra of 3 were indicative of the presence of a trisubstituted double bond between C-10 and C-11 and a tertiary alcohol group on C-12 instead of the epoxy group found in 6. These observations, together with the molecular ion at m/z 554 which is four mass units larger than the one for 6, suggested that the structure of manzamine H was 3. Manzamine H (3) was prepared by condensation of ircinal B (2) with tryptamine as described above.

Manzamine J (4) was obtained as a colorless solid. The molecular formula of 4 was suggested to be $C_{36}H_{46}N_4O$ by HREIMS (m/z 550.3660, M^+, Δ −1.2 mmu). This molecular formula was the same as that of manzamine B (6). The UV absorptions (218, 236, 280, 290, 348, and 356 nm)[9] indicated the presence of a β-carboline ring. The IR spectrum suggested the presence of NH/OH (3400 and 3220 cm^{-1}). The ^1H and ^{13}C NMR spectra of 4 were also similar to those of 6 except for the fact that an allylic alcohol group was shown to be present at the C-10–C-12 position instead of the epoxy group of 6. Manzamine J (4) was prepared from manzamine B (6) as described in Scheme II.

Recently, a series of β-carboline alkaloids of the manzamine type have been isolated from marine sponges, e.g., manzamines A–D from *Haliclona* sp.,[2] keramamines A and B (= manzamines A and F, respectively) from *Pellina* sp.,[3] and manzamines E and F from *Xestospongia* sp.[4] Manzamine alkaloids are probably biosynthetically derived from the condensation of tryptamine and aldehydes such as ircinals A (1) or B (2). The biogenesis of the complex polycyclic nonaromatic array of 1 or 2 is, however, still unknown.

Ircinals A (1) and B (2) and manzamines H (3) and J (4) exhibited cytotoxicities against L1210 murine leukemia

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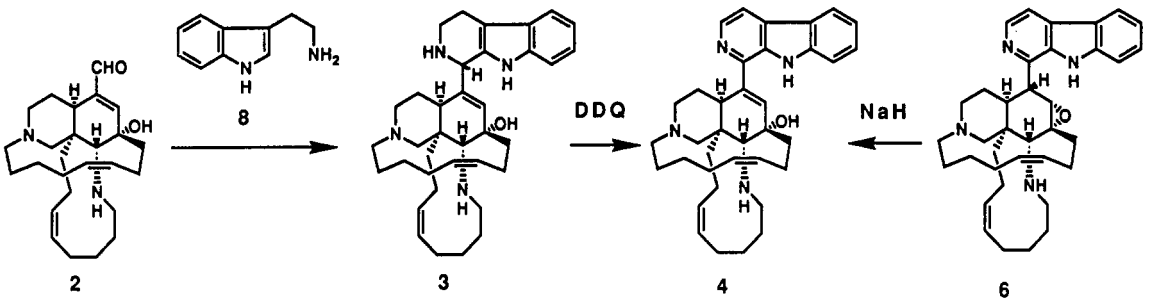
Scheme II



cells with IC_{50} values of 1.4, 1.9, 1.3, and 2.6 μg/mL and KB human epidermoid carcinoma cells with those of 4.8, 3.5, 4.6, and >10 μg/mL in vitro, respectively.

Experimental Section

General Methods. Optical rotations were measured on a DIP-370 polarimeter. UV and IR spectra were taken on a UV-260 and an IR Report-100 spectrometer, respectively. ^1H and ^{13}C NMR spectra were recorded on a JMN GX-270 and an EX-400 spectrometer in $CDCl_3$. The 7.26 ppm resonance of residual $CHCl_3$ and 77.0 ppm resonance of $CDCl_3$ were used as internal references for ^1H and ^{13}C chemical shifts, respectively. Mass spectra were obtained on a DX-303 spectrometer. Wako C-300 silica gel (Wako Pure Chemical) was used for glass column chromatography and TLC was carried out on Merck silica gel GF_{254}. Manzamine alkaloids were detected by Dragendorff's reagent.

Collection, Extraction, and Isolation. The sponge *Ircinia* sp. was collected off Kise, Okinawa, and kept frozen until used. The sponge (1 kg, wet weight) was extracted with methanol (3 L × 2). Evaporation of the extract afforded 76.6 g of residue, which was partitioned between ethyl acetate and 1 M NaCl (500 mL, each). The aqueous layer was extracted with ethyl acetate (500 mL × 3) and then with 1-butanol (500 mL × 3). The ethyl acetate soluble fraction was evaporated under reduced pressure to give a crude residue (7.1 g), which was subjected to a silica gel column (44 × 340 mm) with hexane/acetone (4:1) to give two Dragendorff-positive fractions **a** (680–860 mL) and **b** (1340–2000 mL). Fraction **a** was separated by a silica gel column (23 × 400 mm) with $CHCl_3$/MeOH (95:5) to give two active fractions **a**-1 (400–1410 mL) and **a**-2 (1420–1570 mL). Fraction **a**-1 was further purified by a silica gel column (13 × 400 mm) with hexane/acetone (9:1) to afford ircinal B (**2**, 20 mg, 0.0020% wet weight) in 50–60 mL, ircinal A (**1**, 52 mg, 0.0052%) in 150–270 mL, and manzamine A (**5**, 712 mg, 0.071%) in 390–710 mL. Fraction **a**-2 was separated by a preparative TLC with hexane/acetone (4:1) to afford manzamine J (**4**, 22 mg, 0.0022%). Fraction **b** was further purified by a silica gel column (44 × 330 mm) with $CHCl_3$/MeOH (95:5) to afford manzamine D (**7**, 23 mg, 0.0023%) in 780–980 mL, manzamine B (**6**, 116 mg, 0.012%) in 1280–2000 mL, and manzamine H (**3**, 7 mg, 0.0007%) in 2010–2900 mL.

Ircinal A (1): colorless solid; mp 70 °C; $[\alpha]^{25}_D$ +48° (*c* 2.9, $CHCl_3$); UV (MeOH) λ_{max} 231 nm (ε 8500); IR (KBr) ν_{max} 3420, 2950, 2920, 2850, 2790, 1680, 1670, 1560, 1450, 1400, 1200, 1150, 1100, 1070, and 730 cm^{-1}; ^1H and ^{13}C NMR (Table I); EIMS *m/z* 410 (M^+) 393, 382, and 162; HREIMS *m/z* 410.2924 ((M^+), calcd for $C_{26}H_{38}N_2O_2$, 410.2933).

Ircinal B (2): colorless solid; mp 95 °C; $[\alpha]^{25}_D$ +18° (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} 224 nm (ε 12000); IR (KBr) ν_{max} 3400, 2920, 2850, 2800, 1680, 1450, 1190, 1170, 1120, 1040, and 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.50 (1 H, s), 6.53 (1 H, s) 5.58 (1 H, m), 5.44 (1 H, m), 5.27 (1 H, m), 5.19 (1 H, m), 3.71 (1 H, s), 3.25 (1 H, m), and 3.0–1.0 (complex); ^{13}C NMR ($CDCl_3$) δ 194.3 (d), 151.7 (d), 144.7 (s), 131.4 (d), 131.2 (d), 129.3 (d), 129.2 (d), 69.9 (s), 65.6 (t), 59.8 (d), 59.3 (d), 53.5 (t), 49.5 (t), 42.6 (s), 40.4 (t), 40.2 (d), 37.1 (t), 31.4 (t), 29.2 (t, 4C), 28.6 (t), 26.2 (t), 25.0 (t), and 21.4 (t); EIMS *m/z* 412 (M^+), 395, 383, and 164; HREIMS *m/z* 412.3118 ((M^+), calcd for $C_{26}H_{40}N_2O_2$, 412.3089).

Manzamine H (3): colorless solid; mp 145 °C; $[\alpha]^{27}_D$ +17° (*c*, 1.1 $CHCl_3$); UV (MeOH) λ_{max} 225 (ε 29000), 277 (6600), 282 (6800), and 290 nm (5500); IR (KBr) ν_{max} 3400, 3300, 2990, 2910, 2850, 2780, 1650, 1450, 1360, 1340, 1290, 1260, 1210, 1110, 1070, 1035, 1000, and 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.48 (1 H, d, *J* = 7.6 Hz), 7.33 (1 H, d, *J* = 7.8 Hz), 7.14 (1 H, m), 7.09 (1 H, m), 5.61 (1 H, s), 5.62 (1 H, m), 5.45 (1 H, m), 5.31 (1 H, m), 5.20 (1 H, m), 4.64 (1 H, s), 3.71 (1 H, br s) and 3.4–1.0 (complex); ^{13}C NMR ($CDCl_3$) δ 143.9 (s), 135.5 (s), 134.2 (s), 131.6 (d), 131.1 (d), 130.1 (d), 129.4 (d), 129.1 (d), 127.8 (s), 121.4 (d), 119.3 (d), 118.0 (d), 111.0 (d), 109.0 (s), 70.1 (s), 65.7 (t), 59.9 (d), 59.2 (d), 59.2 (t), 53.4 (t), 49.6 (t), 44.6 (d), 43.5 (s), 43.2 (t), 40.6 (t), 37.3 (t), 32.3 (t), 29.2 (t, 3C), 29.1 (t), 28.6 (t), 26.2 (t), 25.0 (t), 22.4 (t), and 21.9 (t); EIMS *m/z* 554 (M^+), 536, 524, 506, and 164; HREIMS *m/z* 554.3980 ((M^+), calcd for $C_{36}H_{50}N_4O$, 554.3985).

Manzamine J (4): colorless solid; mp 140 °C; $[\alpha]^{25}_D$ +47° (*c* 2.0, $CHCl_3$); UV (MeOH) λ_{max} 218 (ε 26000), 236 (21000), 280 (11000), 290 (11000), 348 (5500), and 356 nm (5600); IR (KBr) ν_{max} 3400, 3220, 2990, 2920, 2850, 2790, 1620, 1560, 1490, 1450, 1420, 1320, 1280, 1230, 1110, 1070, and 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.03 (1 H, br s), 8.44 (1 H, d, *J* = 5.4 Hz), 8.11 (1 H, d, *J* = 7.8 Hz), 7.83 (1 H, d, *J* = 5.1 Hz), 7.53 (2 H, m) 7.27 (1 H, m), 6.24 (1 H, s), 5.64 (1 H, br t, *J* = 11.1 Hz), 5.46 (1 H, br td, *J* = 11.1, 3.8 Hz), 5.36 (1 H, br t, *J* = 9.9 Hz), 5.25 (1 H, br t, *J* = 10.7 Hz), 3.85 (1 H, br s) and 3.33 (1 H, br d, *J* = 11.0 Hz); ^{13}C NMR ($CDCl_3$) δ 144.1 (s), 142.5 (s), 140.2 (s), 138.6 (d), 133.8 (s), 131.7 (d), 131.2 (d), 131.0 (d), 129.44 (d), 129.35 (d, s), 128.2 (d), 121.9 (s), 121.6 (d), 120.0 (d), 113.4 (d), 111.7 (d), 70.4 (s), 65.6 (t), 59.2 (d, t), 53.5 (t), 49.7 (t), 46.7 (d), 43.5 (s), 40.9 (t), 37.5 (t), 32.5 (t), 29.2 (t, 4C), 28.7 (t), 26.2 (t), 25.1 (t), and 22.2 (t); EIMS *m/z* 550 (M^+), 532, 517, and 164; HREIMS *m/z* 550.3660 ((M^+), calcd for $C_{36}H_{46}N_4O$, 550.3672).

Pictet–Spengler Cyclization of Ircinal A (1) with Tryptamine (8). To a stirred solution of ircinal A (1, 4.0 mg) and tryptamine (8, 2.3 mg) in toluene (0.6 mL) at room temperature was added trifluoroacetic acid (20 μL). The mixture was stirred at room temperature for 6 h. After evaporation of the solvent, the residue was passed through a silica gel column (0.5 × 5 cm) with $CHCl_3$/MeOH (95:5) to afford compound **7** (= manzamine D, 1.0 mg). A total of 2.0 mg of **1** was recovered. **7**: yellowish solid; mp 160 °C; $[\alpha]^{29}_D$ +29° (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} 224 (ε 20000) and 283 nm (4600); IR (film) ν_{max} 3220, 3000, 2930, 2850, 2800, 1670, 1650, 1455, 1370, 1315, 1300, 1260, 1220, 1150, 1075, 1020, 800, and 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.04 (1 H, br s), 7.78 (1 H, d, *J* = 5.1 Hz), 7.66 (1 H, d, *J* = 7.0 Hz), 7.26 (2 H, m), 6.91 (1 H, s), 5.91 (1 H, m), 5.77 (1 H, s), 5.66 (2 H, m), 5.14 (1 H, m), 4.59 (1 H, s), 4.22 (1 H, m), and 3.44 (1 H, br s); EIMS *m/z* 552 (M^+), 534, 440, 399, 379, and 162.

DDQ Oxidation of Compound 7. To a stirred solution of **7** (12.9 mg) in EtOH (0.3 mL) and $CHCl_3$ (0.8 mL) at room temperature was added DDQ (2,3-dichloro-5,6-dicyanobenzoquinone, 8.4 mg). The mixture was stirred for 30 min. After evaporation of the solvent, the residue was passed through a silica gel column (0.5 × 5 cm) with hexane/acetone (4:1) to afford manzamine A (**5**, 5.6 mg). A total of 2.5 mg of **7** was recovered. $[\alpha]_D$, UV, IR, ^1H NMR, and EIMS data were identical with those of authentic sample.

Pictet–Spengler Cyclization of Ircinal B (2) with Tryptamine (8). To a stirred solution of ircinal B (**2**, 3.6 mg) and tryptamine (8, 2.1 mg) in EtOH (0.1 mL) and $CHCl_3$ (0.5 mL) at room temperature was added trifluoroacetic acid (5 μL). The mixture was stirred at room temperature for 3 days. After evaporation of the solvent, the residue was passed through a silica gel column (0.5 × 5 cm) with $CHCl_3$/EtOH (95:5) to afford

manzamine H (3, 0.9 mg). A total of 0.8 mg of 2 was recovered.

DDQ Oxidation of Compound 3. To a stirred solution of 3 (4.3 mg) in EtOH (0.1 mL) and $CHCl_3$ (0.3 mL) at room temperature was added DDQ (2.8 mg). The mixture was stirred for 30 min. After evaporation of the solvent, the residue was passed through a silica gel column (0.5 × 5 cm) with $CHCl_3$ to afford manzamine J (4, 1.6 mg).

Treatment of Manzamine B (6) with NaH. To a stirred solution of 6 (10.0 mg) in DMF (1 mL) at room temperature was added NaH (7.1 mg). The mixture was refluxed for 12 h. To the reaction mixture was added 1 N HCl/MeOH (0.25 mL). After evaporation of the solvent, the residue was passed through a silica gel column (0.5 × 5 cm) with $CHCl_3$ to afford compound 4 (3.0 mg). A total of 4.2 mg of 6 was recovered.

Biological Assay. Antitumor activity was determined by using L1210 murine leukemia cells and KB human epidermoid carcinoma cells according to the method previously reported.[10]

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Supplementary Material Available: All spectra of ircinals A and B and manzamines H and J (39 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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Study of 1,2-Chlorine Migration in (α,α-Dichlorobenzyl)chlorocarbene Generated by Laser Flash Photolysis of 3-Chloro-3-(α,α-dichlorobenzyl)diazirine

Michael T. H. Liu*

Department of Chemistry, University of Prince Edward Island, Charlottetown, P.E.I., Canada C1A 4P3

Roland Bonneau

URA 348 du CNRS, Laboratoire de Chimie Physique A, Universite de Bordeaux 1, 33405 Talence Cedex, France

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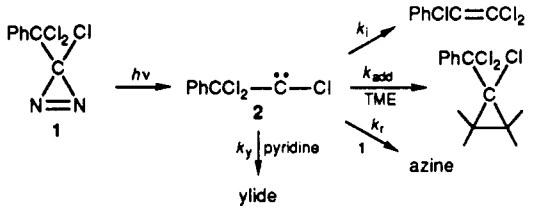
Although the literature is replete with information[1-5] on 1,2-hydrogen shifts in carbenes, few investigations involving chlorine migration to a carbene center are reported. By employing the laser flash photolysis (LFP) technique and the appropriate carbene precursors, the rates for 1,2-hydrogen shift in benzyl-[6] and alkylchorocarbenes[7] have been reported recently. We now report the LFP of 3-chloro-3-(α,α-dichlorobenzyl)diazirine. The results that we obtained lead to the rate of a 1,2-chlorine shift[8] in (α,α-dichlorobenzyl)chlorocarbene, 2. The rate constants for the addition of carbene 2 to tetramethylethylene (TME) and diazirine 1 were obtained as well.



Results and Discussion

In the case of benzylchlorocarbene,[6] it was possible to observe directly the transient absorption (carbene) in the 280–330-nm window. However, since the extinction coefficient for diazirine 1 is very low at the exitation wavelength, we use a high concentration of diazirine (0.22 M) for the LFP experiments. As a result, the window at 280–330 nm is not transparent for the observation of the carbene. Similar to the benzylchlorocarbene, the LFP of 1 in isooctane in the presence of pyridine produces a transient species with an absorption at 370 nm. This transient is not present in the absence of pyridine and is attributed to the pyridinium ylide. Normally, the rate of formation of the ylide allows the determination of the absolute rate constant for the 1,2-shift in carbene by plotting the observed pseudo-first-order rate constants for growth of the absorption at 370 nm vs [pyridine]. The slope gives the rate constant for reaction of the carbene with pyridine, and the intercept, [pyridine] = 0, yields the rate constant for 1,2-shift. Upon the addition of a small amount (0.62 mM) of pyridine in a 0.22 M diazirine solution, the growth of the transient at 370 nm is already 20 ns. Therefore, an alternative Stern–Volmer analysis for the amount of ylide is sought for the determination of the rate constant for 1,2-chlorine shift. Also, the quenching reaction of carbene by diazirine to form azine is included in the scheme since this reaction has been recently noted by us[9] and by other investigators.[10,11] k_1 is the rate constant representing the sum of all first-order and pseudo-first-order decay pathways of the carbene in the absence of pyridine, $k_1 = k_i + k_r[1]$. The yield for ylide formation is given by $\phi_{ylide} = k_y[py]/(k_1 + k_y[py])$ and the amount of carbene produced in a single pulse is [carbene] = αE, where E is the reading of the energy meter monitoring the

carbene (2) → styrene, k_i

carbene (2) + diazirine → azine, k_r

carbene (2) + pyridine → ylide, k_y

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