Table I

product	yield, %	reactn time, h
bromobenzene	89	1.0
m-bromonitrobenzene	50	3.0
m-bromobenzoic acid	79	2.5
ethyl *m*-bromobenzoate	80	2.5
methyl *m*-bromobenzoate	78	2.5
m-bromobenzaldehyde	25	1.0
m-bromobenzonitrile	68	2.5
1,4-dibromobenzene	76	2.5
1-bromo-4-chlorobenzene	70	2.5

selective monobromination of reactive aromatic rings. However, no reliable, mild, and selective method exists for the monobromination of deactivated aromatic rings, except some examples[5] where monobromination can be effected, but each example has its own specific set of conditions. The present paper describes a simple method of general applicability for the monobromination of deactivated aromatic rings.

A mixture of mercuric oxide and bromine has been used as brominating reagent for saturated hydrocarbons,[6] but its utility for the aromatic rings has been reported to offer no advantage over the conventional methods. On the contrary, we have found that a mixture of mercuric oxide and bromine in the presence of concentrated sulfuric acid as a catalyst in carbon tetrachloride can be effectively used for the selective monobromination of deactivated aromatic substrates. The products (Table I) were identified by their melting points/boiling points and checked for purity by ^{1}H NMR spectra.

All reactions proceeded smoothly under reflux and gave good to excellent yields of the products. The same monobromo derivatives can also be obtained in slightly diminished yields by the prolonged (24–36 h) stirring of the reaction mixture at ambient temperature. Since no hydrogen bromide is evolved in these reactions, they may be carried out successfully in the open laboratory. The expense of using mercuric oxide as a reagent is compensated by its ease of recovery from mercuric oxide–mercuric bromide residue.[7]

The result with benzoic acid is noteworthy, since carboxylic acids are known to undergo decarboxylation to form bromides (modified Hunsdiecker reaction[8]) with bromine and mercuric oxide, but under our conditions no decarboxylation was observed. In case of esters a minor quantity of *m*-bromobenzoic acid was also isolated along with the desired products. In the reaction of benzaldehyde the main product was *m*-bromobenzoic acid (50%), presumably formed due to oxidation, along with a small quantity (25%) of the *m*-bromobenzaldehyde. These results indicate that the new reagent could be reliably used for the selective bromination of deactivated aromatic substrates.

General Method Used for Bromination. A mixture of the appropriate substrate (0.01 mol), bromine (0.01 mol), mercuric oxide (0.02 mol), concentrated sulfuric acid (1 mL), and carbon tetrachloride (60 mL) was refluxed with vigorous stirring till the reddish brown color of bromine disappeared from the reaction mixture. The reaction mixture was filtered in the hot state and residue extracted with more carbon tetrachloride. Evaporation of the filtrate and extracts gave the crude monobromo derivatives.

Registry No. Benzene, 71-43-2; bromobenzene, 108-86-1; nitrobenzene, 98-95-3; 3-bromonitrobenzene, 585-79-5; benzoic acid, 65-85-0; 3-bromobenzoic acid, 585-76-2; ethyl benzoate, 93-89-0; ethyl 3-bromobenzoate, 6091-64-1; methyl benzoate, 93-58-3; methyl 3-bromobenzoate, 618-89-3; benzaldehyde, 100-52-7; 3-bromobenzaldehyde, 3132-99-8; benzonitrile, 100-47-0; 3-bromobenzonitrile, 6952-59-6; 1,4-dibromobenzene, 106-37-6; chlorobenzene, 108-90-7; 1-bromo-4-chlorobenzene, 106-39-8.

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Cystodytins A, B, and C, Novel Tetracyclic Aromatic Alkaloids with Potent Antineoplastic Activity from the Okinawan Tunicate *Cystodytes dellechiajei*

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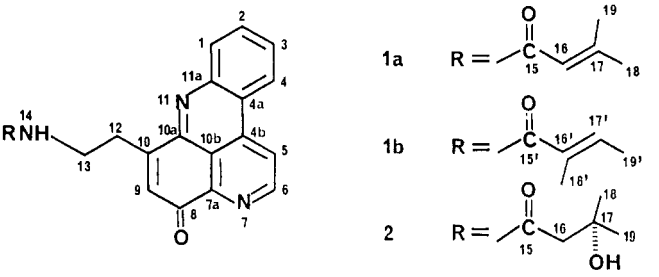
Many alkaloids, most of which exhibit a variety of biological activities, have been isolated from marine plants and animals.[2] During our studies on bioactive substances from tunicates,[3] cystodytins A (**1a**), B (**1b**), and C (**2**), novel



tetracyclic aromatic alkaloids with potent antineoplastic activity and powerful Ca-releasing activity in sarcoplasmic reticulum[3b] have been isolated from the Okinawan tunicate *Cystodytes dellechiajei*.[4] We report here the isolation and structure elucidation of **1a**, **1b**, and **2**. The carbon–carbon connectivities of **1a** and **1b** were unambiguously assigned on the basis of the results of ^{1}H-detected heteronuclear multiple-bond ^{1}H–^{13}C correlation (HMBC) recently reported by Bax.[5] The technique allows us to trace indirectly the complete carbon skeleton of a molecule by $^2J_{CH}$

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and $^3J_{CH}$ connectivities including carbon–hydrogen coupling through nonprotonated carbons and heteroatoms. This method can be useful for a small amount of sample[6] or even an intractable mixture of closely related compounds, since it is more sensitive than the two-dimensional INADEQUATE experiment.

The brown-colored compound tunicate (900 g, wet weight) was collected at Kerama Islands, Okinawa, by SCUBA (−5 to −10 m) and kept frozen until used. The methanol–toluene (3:1) extract of *C. dellechiajei* was partitioned with toluene and water. The toluene-soluble portion, which showed potent cytotoxicity against L1210 murine leukemia cells, was chromatographed on a silica gel column ($CHCl_3/CH_3OH$, 98.5:1.5) followed by a Sephadex LH-20 column ($CHCl_3/CH_3OH$, 1:1) to give a ca. 3.5:1 mixture (1) of cystodytins A (**1a**) and B (**1b**) in 0.022% yield (196 mg) as yellow crystals. It was difficult to separate **1b** from **1a**, since both compounds had the same retention times on HPLC (silica gel or ODS) under several solvent systems. The chloroform extract of the aqueous layer, which exhibited modest cytotoxicity against L1210, was purified by the same procedure as described above to afford cystodytin C (**2**) in 0.0003% yield (2.5 mg) as yellow crystals in addition to 38 mg of 1.

Electron impact mass spectrometry (EIMS) of free base 1 showed mainly the molecular ion at m/z 357 in the beginning of measurement and then a gradual increase in an ion of the reduced form (m/z 359, $M^+ + 2$) to predominate (as observed for quinones[7]). The fast-atom bombardment mass spectrum (FABMS) gave predominantly the ion of the reduced form (m/z 360, $M^+ + 2 + H$) during operation.[8,9] A common molecular formula, $C_{22}H_{19}O_2N_3$, for **1a** and **1b** was established by HRFABMS (m/z 360.1707, Δ −0.5 mmu, $M^+ + 2 + H$) of the reduced form ($C_{22}H_{21}O_2N_3$). The IR bands at 1640 and 1660 cm^{-1} and the ^{13}C signals at δ 167.8–170.3 and 183.2 for 1 indicated the presence of an amide and a conjugated ketone carbonyl group,[10] respectively. The UV spectrum of 1 exhibited absorptions at 225 (ε 35 000), 272 (25 000), and 380 (14 300) nm. In the ^1H NMR spectra of 1, the signals of **1a** accompanied by **1b** in a ratio of 3.5 to 1 were observed, namely for each of them six aromatic (δ 7.6–8.8) and two olefinic protons (δ 5.5–6.7), four protons (δ 3.0–3.6) due to vicinal methylenes, and six protons (δ 1.5–2.0) of two methyl groups attached to a double bond. The RCT-COSY[11] spectrum of **1a** revealed cross peaks of H-5 to H-6, H-9 to H_2-12 and H_2-13, H_2-12 to H_2-13, H-16 to H_3-18 and H_3-19, H_3-18 to H_3-19, and among H-1–H-4, respectively. The ^1H–^{13}C COSY experiment provided definitive assignments for all protonated carbons as shown in Table I. The partial structures of **1a**, -CH=CH- (C-5–C-6), -CH=CHCH=CH- (C-1–C-4), $-CH=CCH_2CH_2-$ (C-9–C-10 and C-12–C-13), and $-CH=C(CH_3)_2$ (C-16–C-19) were thus deduced by interpretation of the ^1H and ^{13}C chemical shifts and ^1H–^1H coupling constants (Table I) as well as the RCT-COSY and the ^1H–^{13}C COSY data. Irradiation of H-12 resulted in a NOE enhancement (16%) of H-9, suggesting that 12-CH_2 was located at cis position to H-9. Further information

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

positn	^{13}C	^1H	J_{HH} (Hz)	HMBC (^1H)
1	131.6	8.07 dd	8.2, 1.4	H-3
2	131.7	7.76 ddd	1.3, 8.2, 8.1	H-4
3	129.8	7.64 ddd	1.4, 8.1, 8.1	H-1
4	122.8	8.30 dd	1.3, 8.1	H-2
4a	121.3			H-1, H-3, H-5
4b	136.9			H-4, H-6
5	119.4	8.22 d	5.5	H-6
6	149.0	8.81 d	5.5	H-5
7a	145.8			H-6, H-9
8	183.2			
9	132.0	6.65 s		H-12
10	152.4			H-12, H-13
10a	149.8			H-9, H-12
10b	117.5			H-5
11a	145.0			H-1, H-2, H-4
12	31.3	3.08 t	6.4	H-9, H-13
13	38.4	3.59 t	6.4	H-12
14		6.01 br s[b]		
15	167.8			H-13, H-16
16	118.1	5.50 qq	1.3, 1.4	H-18, H-19
17	150.8			H-18, H-19
18	26.7	1.65 d	1.4	H-16, H-19
19	19.4	1.93 d	1.3	H-16, H-18

[a] Spectra recorded on Bruker AM-400 spectrometer in $CDCl_3/CD_3OD$ (2:1). [b] Recorded in $CDCl_3$.

on the structural framework was obtained by analyzing the ^1H–^{13}C long-range couplings ($^2J_{CH}$ and $^3J_{CH}$) observed through HMBC experiments of 1 (18 mg in $CDCl_3/CD_3OD$, 2:1) as shown in Figure 1. The cross peak between H-16 and C-15, the higher field resonance (δ 118.1), and the smaller $^1J_{CH}$ (135.0 Hz) of C-16 suggested that C-16 should be attached to the carbonyl group of CONH (C-15 and N-14) but not to the nitrogen, constituting a β,β′-dimethylacryloyl amide unit[12] (N-14–C-19). The C-13 was connected to N-14, since a cross peak was observed between H-13 and C-15 in the HMBC spectrum. This connectivity was also supported by observations of the EIMS fragments at m/z 273, 260, and 247, corresponding to loss of C_5H_8O, C_5H_7NO, and C_6H_8NO from the molecular ion (m/z 357, $C_{22}H_{19}O_2N_3$), respectively. Thus, the partial structure of C-9–C-10 and C-12–C-19 was established.

The presence of a benzene ring (C-1–C-4a and C-11a) was confirmed by cross peaks of H-1 to C-3, C-4a and C-11a, H-2 to C-4 and C-11a, H-3 to C-1 and C-4a, and H-4 to C-2 and C-11a in the HMBC spectrum (Figure 1). The presence of a pyridine ring (C-4b–C-7a and C-10b) was deduced by cross peaks of H-5 to H-10b and H-6 to C-4b and C-7a. Insertion of N-7 between C-6 and C-7a was based on observations of the lower field resonances of C-6 (δ 149.0) and C-7a (δ 145.8) and the larger $^1J_{CH}$ value (183.0 Hz)[13] of C-6. The connectivity of C-4a to C-4b was revealed by cross peaks of H-5 to C-4a and H-4 to C-4b (Figure 1). The relatively lower field resonance of C-10 (δ 152.4) to that of C-9 (δ 132.0) indicated that the conjugated ketone (δ 183.2, C-8) should be placed at this position. This connectivity was supported by the observation of a cross peak of H-9 to C-7a (Figure 1) and confirmed by the structure of the methylated product (**3**) of 1 (vide infra). The presence of a C=N group (C-10a and N-11) was suggested by the chemical composition. The

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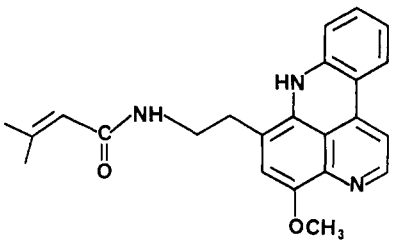

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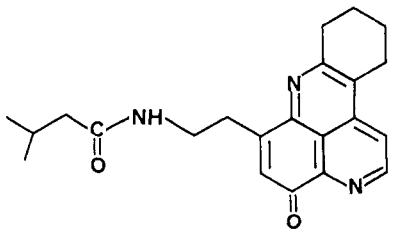
imino carbon (C-10a) was attached to C-10 by the presence of cross peaks of H-9 and H-12 to C-10a, while the imino nitrogen (N-11) was connected to C-11a based on the lower field resonance (δ 145.0) of C-11a and no cross peak of H-1 to other carbons than C-3, C-4a, or C-11a in the HMBC spectrum. Finally, the remaining bond C-10a–C-10b was the only possible way to complete the structural assignment of **1a**.

The well-resolved ^1H and ^{13}C NMR spectra of **1** allowed us to assign the structure of **1b**. Almost the same ^1H and ^{13}C signals for **1b** as those of **1a** were observed except for C-16′–C-19′. The lower field chemical shift (δ 6.19) and the quartet–quartet coupling pattern (J = 1.5 and 6.9 Hz) of H-17′ indicated the presence of an α,β-dimethylacryloyl moiety (C-15′–C-19′) in the side chain of **1b**. The higher field chemical shift (δ 1.51) of H_3-19′ relative to that (δ 1.60) of H_3-18′ implied the double bond of E configuration.[14] This assignment was supported by the higher field chemical shift of C-18′ (δ 11.9).[15] The structure of **1b** was also established by the HMBC spectrum (C-1′–C-19′ in Figure 1), in which were observed cross peaks of H-17′ to C-15′, H_3-18′ to C-15′, each of H_3-18′ and H_3-19′ to both C-16′ and C-17′, respectively.

Methylation of **1** with diazomethane afforded the monomethyl derivative **3** (FABMS, m/z 374, $M^+ + H$) as a major product. The ^1H NMR spectrum ($CDCl_3$) of **3** showed two NH proton signals at δ 6.01 (14-NH) and 10.5 (11-NH) and one methyl group at δ 4.98. This lower field methyl resonance was assigned to an O-methyl group at C-8, since irradiation of this methyl signal resulted in NOE enhancement of H-9 and the carbonyl resonance (δ 183.2) of C-8 disappeared in **3**. Hydrogenation of **1** on PtO_2 in acetic acid led to the reduced product, **4** (EIMS, m/z 363 (M^+) and 365 ($M^+ + 2$)) of the benzene ring (C-1–C-4) and the side-chain dimethylacryloyl moiety (C-16–C-17).



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The EIMS of cystodytin C (**2**) showed a molecular ion peak at m/z 375 and an ion of the reduced form at m/z 377. The UV absorption was the same as that of **1**. The ^1H and ^{13}C NMR spectra of **2** differed from those of **1a** only in the partial structures C-16–C-19, namely the β,β-dimethylacryloyl part of **1a** was replaced by $-CH_2C(CH_3)_2OH$ in **2**. Cystodytin C (**2**) was a H_2O adduct of the C-16–C-17 double bond of **1a**, which could be also obtained by acidic hydrolysis (6% HCl, 100 °C, 3 h) of **1a**.

The tunicate *C. dellechiajei* belongs to the same family as that of *Eudistoma* species in which many β-carboline metabolites[3a,b,16] have been found with potent pharmacological activities. Cystodytins are, however, the first alkaloids with a new class of fused tetracyclic aromatic ring systems containing an iminoquinone isolated from the tunicate, although a fused pentacyclic aromatic alkaloid, amphimedine,[17] has been isolated from a marine sponge. The origin and biosynthetic pathway of these metabolites remain to be resolved. Compounds **1** and **2** were potent cytotoxic compounds, exhibiting IC_{50} values of 0.22 and 0.24 μg/mL against L1210, respectively. Both **1** and **2** also showed powerful Ca-releasing activity in sarcoplasmic reticulum, 36 and 13 times more potent than caffeine, a well-known Ca-releaser,[3b] in Ca-releasing activity, respectively.

Experimental Section

General Methods. All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 260-50 infrared spectrophotometer. UV spectra were measured on a JASCO 660 UV/vis spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Bruker AM-400 or AM-500 or JEOL FX-90Q spectrometers in $CDCl_3$–CD_3OD (2:1) or $CDCl_3$. The 7.27-ppm resonance of residual $CHCl_3$ and 76.9-ppm of $CDCl_3$ were used as internal references for ^1H and ^{13}C NMR. Mass spectra were obtained on a Shimadzu GC-MS QP-1000A spectrometer operating at 70 eV (for EI) or a JEOL HX-100 spectrometer (for FAB).

Collection, Extraction, and Separation. *C. dellechiajei*, a brown-colored compound tunicate, was collected at Kerama Islands, Okinawa, in 1984, using SCUBA (−5 to −10 m), frozen, and shipped via air to Tokyo. The tunicate (900 g, wet weight), stored at −20 °C, was cut into small pieces and extracted with methanol–toluene (3:1, 1 L × 2). After addition of 1 M NaCl (1.2 L) and extraction with toluene (500 mL × 4), evaporation of toluene-soluble portion under reduced pressure afforded a crude extract (2.55 g). The aqueous layer was extracted with chloroform (500 mL × 4). The chloroform-soluble fraction was evaporated under reduced pressure to give a crude extract (0.34 g). Separation of the toluene extract by flash chromatography on a silica gel column (Wako gel C-300, Wako Chemical, 3 × 50 cm) eluted with chloroform/methanol (98.5:1.5) afforded an active fraction (820–920 mL), which was subjected to chromatography on a Sephadex LH-20 column (Pharmacia Fine Chemicals, 2.5 × 90 cm) with chloroform/methanol (1:1) to give a 3.5:1 mixture (**1**, 196 mg, 0.022% wet weight) of cystodytins A (**1a**) and B (**1b**). The chloroform extract was purified on a silica gel column (Wako gel C-300, 2.5 × 40 cm) with methanol/chloroform (1.5:98.5) to give an active fraction (950–970 mL), which was applied to a LH-20 column (2.5 × 90 cm) with chloroform/methanol (1:1) to yield cystodytin C (**2**, 2.5 mg, 0.0003% wet weight) and a 3.5:1 mixture (**1**, 38 mg) of **1a** and **1b**.

3.5:1 Mixture (1) of cystodytins A (1a) and B (1b): yellow crystals; mp 181–183 °C; UV (MeOH) λ_{max} 225 (ϵ 35 000), 272 (25 000), and 380 (11 400) nm; IR (KBr) ν_{max} 3290, 2925, 2850, 1660, 1640, 1590, 1520, 1330, 1300, 1180, 860, and 760 cm^{-1}; EIMS, m/z 359 ($M^+ + 2$), 357 (M^+), 328, 273, 260, and 247; HRFABMS, found 360.1707, calcd for $C_{22}H_{22}O_2N_3$ ($M + 2 + H$)$^+$, 360.1712; ^1H and ^{13}C NMR (Table I) for **1a**; ^1H NMR ($CDCl_3/CD_3OD$, 2:1) for **1b** δ 1.51 (dq, 3 H, J = 1.2 and 6.9 Hz, H-19′), 1.60 (dq, 3 H, J = 1.2 and 1.5 Hz, H-18′), 3.11 (t, 2 H, J = 6.4 Hz, H-12′), 3.60 (t, 2 H, J = 6.4 Hz, H-13′), 6.19 (qq, 1 H, J = 1.5 and 6.9 Hz, H-17′), 6.67 (s, 1 H, H-9′), 7.64 (ddd, 1 H, J = 1.7, 8.1 and 8.1 Hz, H-3′), 7.76 (ddd, 1 H, J = 1.3, 8.1 and 8.2 Hz, H-2′), 8.07 (dd, 1 H, J = 1.7 and 8.2 Hz, H-1′), 8.33 (d, 1 H, J = 5.5 Hz, H-5′), 8.34 (dd, 1 H,

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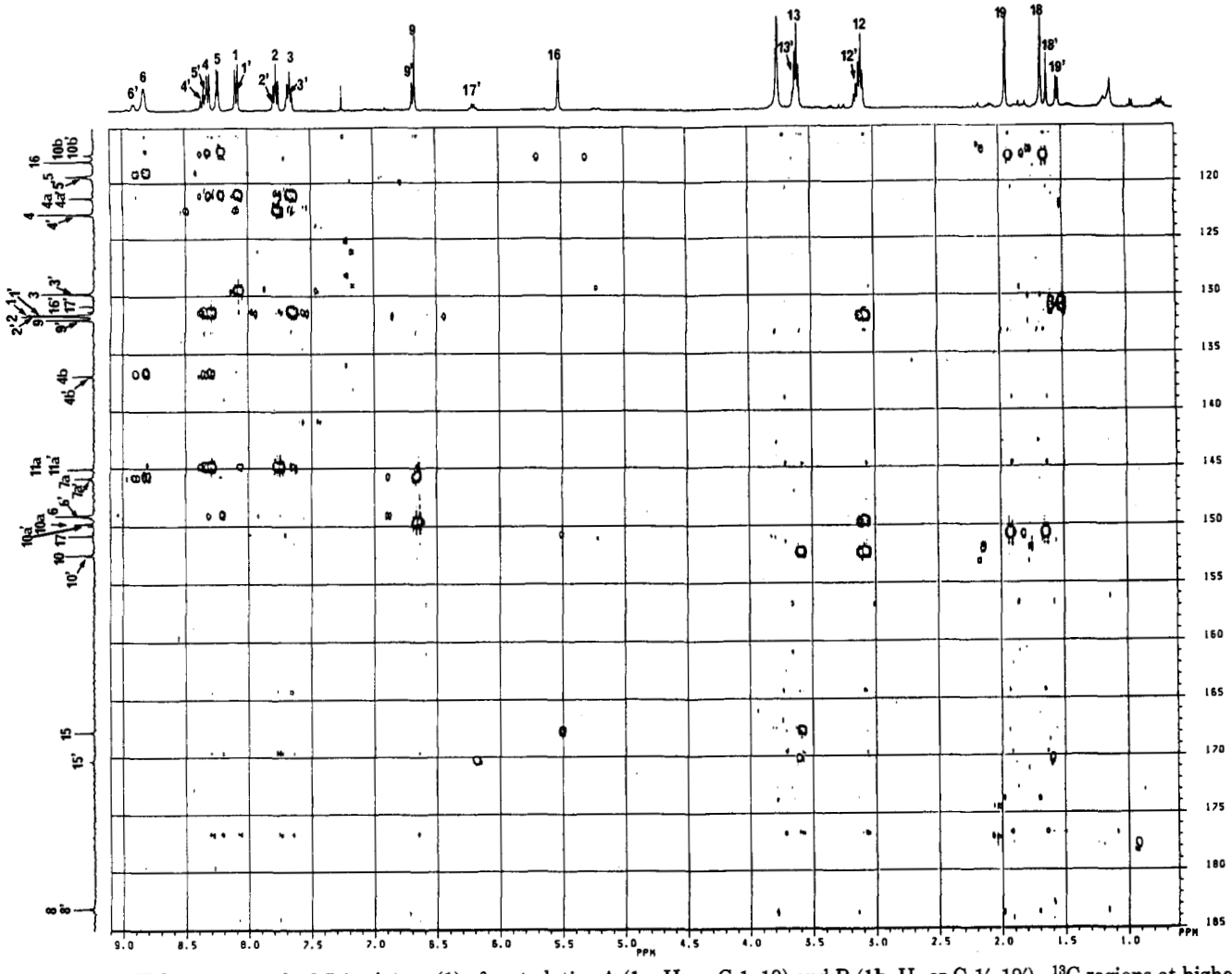

Figure 1. HMBC spectrum of a 3.5:1 mixture (1) of cystodytins A (**1a**, H- or C-1–19) and B (**1b**, H- or C-1′–19′). ^{13}C regions at higher field ($\delta < 115$) are not shown here.

J = 1.3 and 8.1 Hz, H-4′), and 8.89 (d, 1 H, J = 5.5 Hz, H-6′); ^{13}C NMR ($CDCl_3/CD_3OD$, 2:1) for **1b** δ 11.9 (q, C-18′), 13.4 (q, C-19′), 30.9 (t, C-12′), 39.3 (t, C-13′), 117.5 (s, C-10′b), 119.5 (d, C-5′), 121.4 (s, C-4′a), 122.8 (d, C-4′), 129.8 (d, C-3′), 130.0 (d, C-17′), 130.3 (s, C-16′), 131.3 (d, C-1′), 131.8 (d, C-2′), 132.0 (d, C-9′), 137.0 (s, C-4′b), 145.0 (s, C-11′a), 145.9 (s, C-7′a), 149.1 (d, C-6′), 149.9 (s, C-10′a), 152.5 (s, C-10′), 170.3 (s, C-15′), and 183.2 (s, C-8′).

Cystodytin C (2): light yellow crystals; mp 257–259 °C; UV (MeOH) λ_{max} 228 (ϵ 29 900), 272 (29 100), and 380 (11 800) nm; IR (KBr) ν_{max} 3400, 2930, 2850, 1660, 1640, 1580, and 760 cm^{-1}; EIMS, m/z 377 (M^+ + 2), 375 (M^+), 373, 328, 315, 273, 259, and 247; ^1H NMR ($CDCl_3/CD_3OD$, 2:1) δ 1.19 (s, 6 H, H-18 and H-19), 2.29 (s, 2 H, H-16), 3.25 (t, 2 H, J = 6.4 Hz, H-12), 3.75 (t, 2 H, J = 6.4 Hz, H-13), 6.86 (s, 1 H, H-9), 7.81 (dd, 1 H, J = 8.0 and 8.0 Hz, H-3), 7.90 (dd, 1 H, J = 8.0 and 8.2 Hz, H-2), 8.25 (d, 1 H, J = 8.2 Hz, H-1), 8.44 (d, 1 H, J = 5.5 Hz, H-5), 8.50 (d, 1 H, J = 8.0 Hz, H-4), and 9.04 (d, 1 H, J = 5.5 Hz, H-6); ^{13}C NMR ($CDCl_3/CD_3OD$, 2:1) δ 28.9 (q, C-18 and C-19), 31.4 (t, C-12), 38.6 (t, C-13), 47.6 (t, C-16), 65.0 (s, C-17), 117.0 (s, C-10b), 119.5 (d, C-5), 121.1 (s, C-4a), 122.9 (d, C-4), 129.8 (d, C-3), 131.7 (d, C-1 and C-2), 132.4 (d, C-9), 137.2 (s, C-4b), 145.3 (s, C-7a and C-11a), 149.5 (d, C-6), 149.9 (s, C-10a), 153.2 (s, C-10), 172.7 (s, C-15), and 183.5 (s, C-8).

Monomethyl Derivative 3. To an ethereal solution (10 mL) saturated with diazomethane was added a methanol solution (1 mL) of 1 (6.5 mg), which stood at room temperature overnight. The solution was evaporated under reduced pressure and the residue was chromatographed on a silica gel column (Wako gel C-300, 1 × 15 cm) by using methanol/chloroform (3:97) to give the monomethyl derivative 3 (1.5 mg, 23% theoretical yield) as red crystals: mp 172–173 °C; UV (MeOH) λ_{max} 227 (ϵ 26 700), 269 (18 600), 325 (7100), 370 (2900), and 454 (3100) nm; FABMS, m/z 374 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 1.93 (s, 3 H, H-18), 2.34 (s, 3 H, H-19), 2.95 (t, 2 H, J = 6.4 Hz, H-12), 3.34 (m, 2 H, J = 5.5 and 6.4 Hz, H-13), 4.98 (s, 3 H, OMe on C-8), 5.67 (br s, 1 H, H-16), 16.01 (t, 1 H, J = 5.5 Hz, H-14), 7.04 (t, 1 H, J = 8.0 and 8.0 Hz, H-3), 7.24 (s, 1 H, H-9), 7.33 (d, 1 H, J = 5.0 Hz, H-5), 7.42 (t, J = 8.0 and 8.0 Hz, H-2), 7.51 (d, 1 H, J = 8.0 Hz, H-1), 7.90 (d, 1 H, J = 8.0 Hz, H-4), 8.56 (d, 1 H, J = 5.0 Hz, H-6), and 10.5 (br s, 1 H, H-11); ^{13}C NMR ($CDCl_3$) δ 20.0 (q, C-19), 27.2 (q, C-18), 31.8 (t, C-12), 38.9 (t, C-13), 65.5 (q, OMe on C-8), 106.4 (d, C-1), 110.4 (d, C-9), 116.6 (s), 116.8 (d, C-2), 117.5 (d, C-5), 119.2 (s), 120.8 (d, C-4), 123.5 (d, C-3), 124.0 (s, C-4a), 131.2 (s), 131.7 (s, 2 × C), 135.8 (s, C-11a), 140.4 (s), 149.8 (d, C-6), 152.8 (s, 2 × C), and 168.9 (s, C-15).

Hydrogenation Derivative 4. A solution of 1 (6.8 mg) in acetic acid (5 mL) was hydrogenated over PtO_2 for 6 h. The filtrate of the reaction mixture was evaporated under reduced pressure and the residue was subjected to chromatography on a silica gel column (Wako gel C-300, 1 × 10 cm) with methanol/chloroform (2:98) to afford the hydrogenated derivative 4 (5.0 mg, 76% theoretical yield) as yellow crystals: mp 128–132 °C dec; UV (MeOH) λ_{max} 229 (ϵ 31 200), 250 (28 400), 353 (5200), and 411 (7500) nm; IR (KBr) ν_{max} 3500–3000, 2925, 1660, 1640, and 1580 cm^{-1}; EIMS, m/z 365 (M^+ + 2), 363 (M^+), 361, 271, 265, 251, 235, and 218; ^1H NMR ($CDCl_3$) δ 0.88 (d, 6 H, J = 6.3 Hz, H-18 and H-19), 2.01 (m, 7 H, H-2, H-3, H-16, and H-17), 3.15 (m, 6 H, H-1, H-4, and H-12), 3.70 (m, 2 H, H-13), 6.17 (br s, 1 H, H-14), 6.74 (s, 1 H, H-9), 7.90 (d, 1 H, J = 5.8 Hz, H-6), 8.99 (d, 1 H, J = 5.8 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 21.5 (d, C-3), 22.0 (q, C-18 and C-19), 22.1 (t, C-2), 24.1 (t, C-4), 25.6 (d, C-17), 31.1 (t, C-12), 32.7 (t, C-1), 38.7 (t, C-13), 45.7 (t, C-16), 117.8 (s, C-10b), 119.0 (d, C-5), 126.0 (s, C-4a), 130.8 (d, C-9), 137.6 (s, C-4b), 147.1 (s, C-7a), 147.9 (d,

C-6), 147.9 (s, C-11a), 151.7 (s, C-10a), 154.4 (s, C-10), 171.9 (s, C-15), and 183.1 (s, C-8).

Biological Assay. Antitumor activity was determined by using mouse leukemia cell lines L1210 according to the method previously reported.[3c] The extravesicular Ca^{2+} concentration in sarcoplasmic reticulum was monitored with a Ca^{2+} electrode prepared by the method of Tsien and Rink with modifications.[3b]

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Supplementary Material Available: Figures of the HMBC, ^1H-^{13}C COSY, and RTC-COSY spectra of a 3.5:1 mixture (1) of cystodytins A (1a) and B (1b) (4 pages). Ordering information is given on any current masthead page.

Novel Synthesis of the 2,3-Benzindolizine Ring System. Mechanism of Formation, Redox, Electronic Absorption, and Fluorescence Behavior

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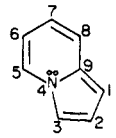
Synthesis of the indolizine ring system 1 has been previously accomplished by the reaction of pyridine with diphenylcyclopropenone by Breslow,[1] Lown,[2] and Wadsworth.[3] In this reaction pyridine and a cyclopropenone condense to form the indolizine nucleus. Indolizines have



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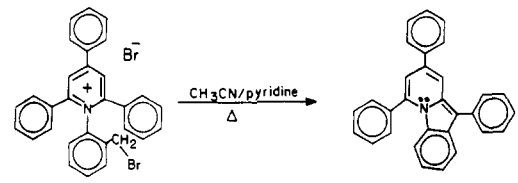
recently formed the basis for the synthesis of novel dye classes which have found application in imaging.[4]

We report a novel synthesis of 2,3-benzindolizines by the pyridine-catalyzed reaction of phenyl-substituted pyridinium derivatives via a 1,2-phenyl migration.

Compound 2 was synthesized in a straightforward manner by the reaction of HBr or thionyl bromide with the product of the condensation of triphenylpyrylium chloride with 2-(hydroxymethyl)aniline. The triphenylbenzindolizine 3 was obtained in 83% yield when the pyridinium derivative 2 was heated at reflux in acetonitrile in the presence of pyridine or its derivatives. Triethylamine was found not to be effective at catalyzing the transformation from 2 to 3. The latter are required for the reaction. The structure of 3 was confirmed by its X-ray crystal structure.

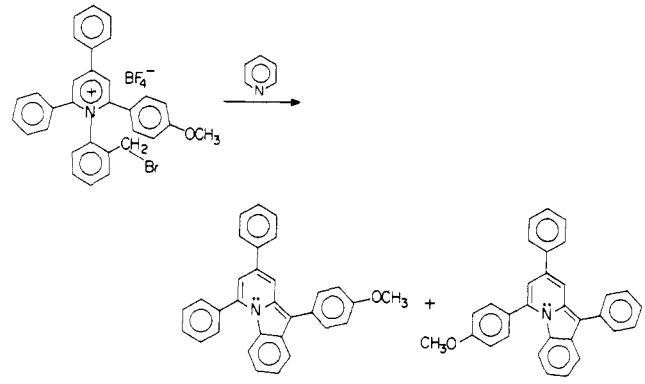
The pyridinium-to-indolizine transformation requires a 1,2-phenyl migration subsequent to ring closure of the



2 3

benzylic carbon on the pyridinium ring. Scheme I shows the reactions proposed to account for the observed chemical transformation.

The proposed mechanism involves an initial substitution reaction to form a benzylpyridinium salt which activates the benzylic carbon to deprotonation by pyridine. The pyridinium ylide adds to the triphenylpyridinium ring before loss of pyridine to produce a resonance-stabilized carbocation. The carbocation then undergoes a fast stepwise 1,2-phenyl shift followed by deprotonation to form the indolizine 3. The 4-methoxyphenyl derivative (6) was synthesized from the corresponding hydroxymethyl precursor (5) in order to determine if there was a preference for *p*-anisyl over phenyl migration. Compound 6 was heated at reflux in pyridine for 2 h before purification of the benzindolizine fraction by column chromatography on silica gel with cyclohexane as eluant. Thin layer chromatography on silica gel showed a single spot while field desorption mass spectrometry (FDMS) showed an intense signal corresponding to the expected indolizine (m/e = 425). In addition, ^1H NMR showed the presence of two methoxy signals in a 1:1 ratio, indicating an equal molar mixture of 7 and 8. Cyclic voltammetry showed the mixture to possess a single reversible oxidation wave at +0.64 V (vs SCE in CH_2Cl_2), which produces a cathodic wave at −0.86 V (E_p at 200 mV/s) at slow scan rates. The redox behavior and ^1H NMR spectrum of the mixture of 7 and 8 is entirely consistent with that observed for 3 and 4.



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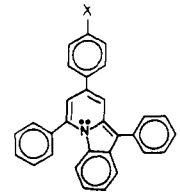
Starting with a [4-(dimethylamino)phenyl]pyrylium derivative, 7-[(dimethylamino)phenyl]indolizine (4) was also synthesized. The redox behavior, electronic absorption, and fluorescence behavior of the two benzindolizines (3 and 4) have been investigated.



3 X = H

4 X = -N(CH$_3$)$_2$

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