Kinetics of Alkylation Reactions of Pyrrolizidine Alkaloid Pyrroles

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Received June 17, 1986

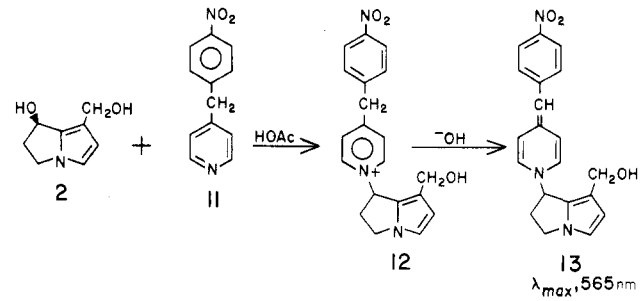
Pyrrolizidine alkaloid pyrroles react rapidly with 4-(*p*-nitrobenzyl)pyridine under pseudo-first-order conditions. A biexponential first-order rate law is observed. Relative reactivities are greatly influenced by the nature of the leaving group at C7. 2,3-Dihydro-1*H*-pyrrolizin-1-ol and dehydrosupinidine under pseudo-first-order reaction conditions obey a simple first-order rate expression. Evidence for oligomerization of the pyrrolizidine pyrrole nucleus has been obtained under these reaction conditions, and it is proposed that a second color-forming reaction sequence of nucleophile with the oligomer C9s accounts for the biexponential rate law.

Introduction

Pyrrolizidine alkaloids are widely distributed throughout the world in a variety of plant species. Hepatotoxic effects in cattle and horses from ingestion of plants containing pyrrolizidine alkaloids has been a concern to stockmen for many years.[1,2] Only recently has the unintentional exposure of humans through contamination of food sources become a potential public health issue.[3,4]

Retronecine (1) (Chart I), referred to as a necine base, may be esterified with various necic acids[5] to make up many naturally occurring pyrrolizidine alkaloids. An important structural feature required for toxicity is the presence of a 1,2-double bond, but the toxic action of these alkaloids is probably exerted through reactive pyrrole derivatives **2–10**.[6] These alkaloids have been shown to be carcinogenic[7] and mutagenic[8] and their pyrrolic metabolites are known to react with DNA[9] and other cellular nucleophiles.[10] Recently it was shown that dehydroretronecine (**2**) reacts with purine and pyrimidine nucleosides and nucleotides.[11] Earlier investigations showed that the pyrroles react rapidly with 4-(*p*-nitrobenzyl)-pyridine (**11**) under pseudo-first-order reaction conditions and that the rate of alkylation can be followed colorometrically at 565 nm after quenching the reaction with base (Scheme I).[12] Further studies have been carried out on the reactions of these pyrroles which we wish to report at this time.

Scheme I



Experimental Section

Instrumentation. ^{1}H NMR spectra were recorded on a Bruker AM 400 spectrometer at 400.6 MHz using a 5-mm multinuclear probe. NMR spectra are reported as parts per million downfield from Me_4Si (δ 0.0).

Mass spectra were obtained on a Varian CH-7 single-focusing instrument equipped with a saddle field Ion Tech Ltd. fast atom bombardment (FAB) source. Xenon atoms with a mean energy of 7 keV were used for sputtering. The matrix consisted of a dithiothreitol–erythritol (magic bullet) 5:1 mixture or glycerol.

Dehydroretronecine (2). Retronecine (1), which was prepared by hydrolysis of monocrotaline,[13] was dehydrogenated with chloranil[14] to give dehydroretronecine (**2**). The crude product was purified by sublimation and recrystallization from hexane–acetone, mp 91 °C (lit.[14] mp 91–93 °C). NMR and mass spectral data were consistent with that reported in the literature. NMR (400 MHz, $CDCl_3$) δ: 6.54 d (H3); 6.16 d (H2); 5.24 m (H7); 4.68 d, 4.57 d (H9); 4.13 m, 3.89 m (H5); 2.79 m, 2.43 m (H6).

2,3-Dihydro-1*H*-pyrrolizin-1-ol (3). Cyanoethylation of pyrrole[16] with subsequent cyclization of the intermediate nitrile using the procedure of Josey and Jenner[17] produced 2,3-dihydro-1*H*-pyrrolizin-1-one. This product (200 mg) was allowed to react with sodium borohydride (47 mg) in water (15 mL), and the reaction was monitored by thin-layer chromatography (TLC) [SiO_2, diethyl ether]. The ketone had R_f 0.72, and the alcohol had R_f 0.56. The ketone was detected by UV and the alcohol by 5% *p*-toluenesulfonic acid in ethanol spray reagent, which gave an orange-colored spot after a few minutes. After 4 h the TLC spot for the starting material was absent. The reaction mixture was then extracted three times with an equal volume of diethyl ether, and the combined ethereal extracts were dried over sodium sulate, filtered, and evaporated to give 190 mg of a white crystalline material. Final purification by sublimation [35 °C (0.025 mmHg)] gave 152 mg of product as white crystals: mp 66–67 °C (lit.[18] oil); NMR (100 MHz, $CDCl_3$:) δ 6.55 (1 H, dd, $J_{5,6}$ = 2.5 Hz, $J_{5,7}$ = 1.2 Hz, H5), 6.18 (1 H, t, $J_{5,6}$ = 2.5 Hz, $J_{6,7}$ = 3.5 Hz, H6), 6.0 (1 H,

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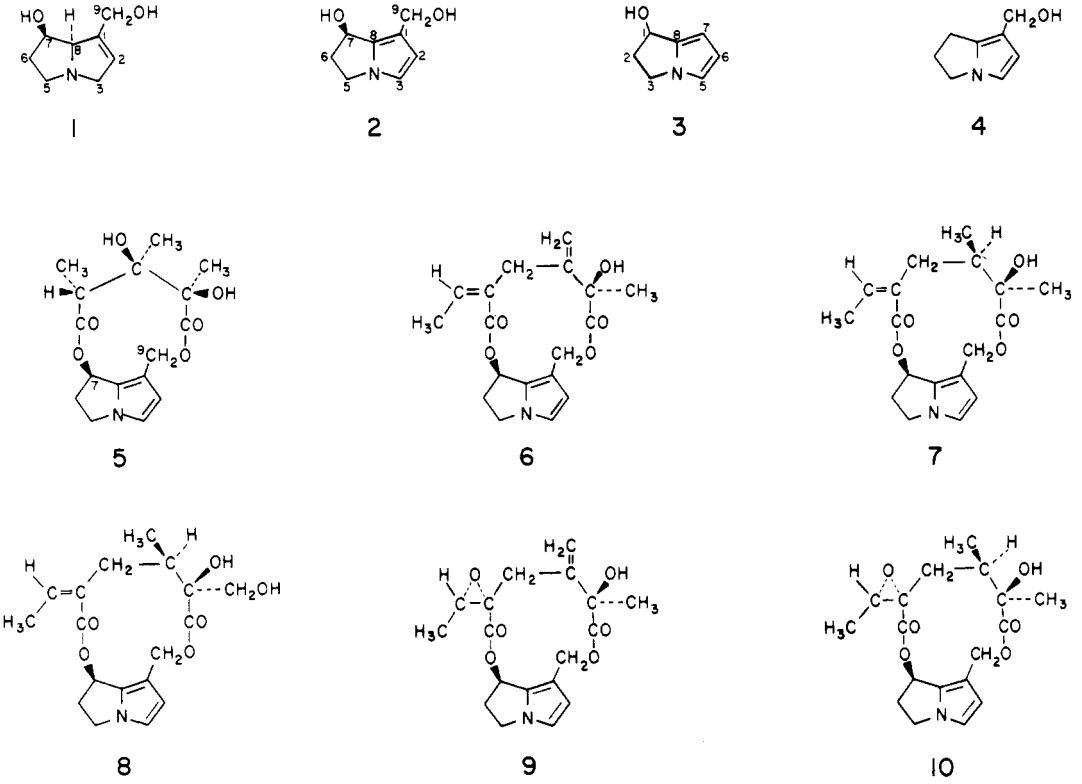
Chart I



dd, $J_{6,7}$ = 3.5 Hz, $J_{5,7}$ = 1.2 Hz, H7), 5.10 (1 H, dd, J = 2 Hz and 6 Hz, H1), 4.0 (2 H, m, H3), 2.60 (2 H, m, H2), 1.76 (1 H, s, OH); MS (EI-MS), m/z (relative intensity) 123 ($M^{\cdot+}$, 97), 106 (100, M - OH), 94 (77); high-resolution mass spectrum was consistent with the structure, $M^{\cdot+}$ = 123.068 (calcd 123.0684).

Dehydrosupinidine (4). *N*-Formylproline and ethyl propiolate were allowed to react according to the procedure of Pizzorno and Albonico.[19] The ester produced from the cycloaddition reaction was purified by reverse-phase HPLC (Whatman C-8; methanol–water, 45:55). The purified product was subjected to reduction, using excess Vitride in refluxing benzene for 30 min. Dehydrosupinidine (**4**) was obtained as an oil. Its NMR spectrum was consistent with published data.[14] The high-resolution mass spectrum was consistent with the structure, $M^{\cdot+}$ = 137.084 (calcd 137.0841).

Pyrrolizidine Alkaloid Pyrroles 5–10. Monocrotaline pyrrole (**5**), seneciphylline pyrrole (**6**), senecionine pyrrole (**7**), retronecine pyrrole (**8**), jacozine pyrrole (**9**), and jacobine pyrrole (**10**) were synthesized from their respective parent macrocyclic pyrrolizidine alkaloids. The macrocyclic pyrrolizidine alkaloids were converted first to their *N*-oxides using hydrogen peroxide in methanol as described by Mattocks.[12a] The *N*-oxides were then converted to the pyrroles by use of acetic anhydride in $CHCl_3$ as described by Culvenor et al.[14] Typically in this case, 50 mg of *N*-oxide was allowed to react with 0.04 mL of acetic anhydride in 10 mL of $CHCl_3$ for 15–30 min. The $CHCl_3$ solution was extracted with a large excess of dilute sodium bicarbonate, followed by water, then dried with anhydrous sodium sulfate, and filtered. The filtered $CHCl_3$ solution was added to 50 mL of anhydrous ether and stirred with a small amount of DOWEX-I for 2–5 min, then filtered, and evaporated under nitrogen. The pyrrole was further dried under vacuum for 2–4 h or until an NMR spectrum showed no trace of residual acetic anhydride. The pyrroles **5–10** all exhibited NMR and mass spectra consistent with their structures.[12a,14]

The parent pyrrolizidine alkaloids were obtained as follows. Monocrotaline was purchased from Trans World Chemicals, P.O. Box 4173, Washington, DC 20015. Jacobine, jacozine, senecionine, and seneciphylline were isolated from *Senecio jacobea L.* and retrorsine as well as senecionine and seneciphylline were isolated from *Senecio vulgaris L.* by a cold ion-exchange extraction procedure.[15] Final purification was accomplished by preparative HPLC on a Whatman C-8 reversed-phase column (methanol–0.1 M pH 6.0 phosphate buffer, 42:58, v/v; 40 °C) and subsequent recrystallization from methanol to give compounds with melting points, NMR, and mass spectra consistent with published data.[1,20] Purity of the recrystallized pyrrolizidine alkaloids was checked by GC–MS. Each compound exhibited a single and distinct GC peak.

NMR Spectra of Pyrrolizidine Alkaloids. Jacobine. NMR (400 MHz, $CDCl_3$, C. = centered at) δ: 6.21 (H2); 5.57 d, 4.06 d (H9); 5.12 m (H7); 4.28 m, (H8); 3.98 d, 3.39 dd (H3); C. 3.30 m, C. 2.45 m (H5); 2.95 q (H, epoxide); 2.25 dd, C. 2.14 m (H6); 2.02 d, 1.11 q ($CH_2(CH_2CHCH_3)$); 1.95 m (H-CCH_3); 1.33 s (CH_3(C-H_3COH)); 1.23 d ($CH_3(CH_3)$, epoxide); 1.48 d ($CH_3(CH_3CH)$).

Seneciphylline. NMR (400 MHz, $CDCl_3$) δ 6.19 (H2); 5.85 q (H, H(CH_3)C=C<); 5.41 d, 4.03 d (H9); 5.25 d, 5.06 d (CH_2=); 4.97 m (H7); 4.24 m (H8); 3.94 d, 3.41 dd (H3); 3.26 m, C. 2.55 m (H5); 2.96 d, 2.76 d ($CH_2(CH_2C$=CH_2); 2.35 dd, C. 2.1 (H6); 1.88 ($CH_3(CH_3(CH_3CH$=$C)))$; 1.55 s ($CH_3(CH_3COH)$).

Senecionine. NMR (400 MHz, $CDCl_3$) δ: 6.20 (H2); 5.72 q (H, H(CH_3)C=C<); 5.50 d, 4.06 d (H9); 5.02 m (H7); 4.29 m (H8); 3.95 d, 3.41 dd (H3); 3.29 m, C. 2.54 m (H5); 2.38 dd, C. 2.10 m (H6); C. 2.18 m, C. 1.75 m ($CH_2(CH_2CHCH_3)$); 1.86 m (CH_3(C-H_3CH=C)); C. 1.67 m (H($HCCH_2$)); 1.33 s ($CH_3(CH_3COH)$); 0.92 d ($CH_3(CH_3CH)$).

Retrorsine. NMR (400 MHz, $CDCl_3$) δ 6.22 (H2); 5.72 q (H, H(CH_3)C=C<); 5.51 d, 4.29 d (H9); 5.02 m (H7); 4.29 m (H8); 3.96 d, 3.41 dd (H3); 3.76 d, 3.65 d ($CH_2(CH_2OH)$); 3.28m C. 2.55m (H5); 2.41d, C. 2.15m (H6); C. 2.21m, C. 1.76m ($CH_2(CH_2CHCH_3)$); C. 1.85 d ($CH_3(CH_3CH$=C)); 0.867 d ($CH_3(CHCH_3)$).

Monocrotaline. NMR (400 MHz, $CDCl_3$) δ: 6.04 (H2); 5.05 m (H7); 4.89 d, 4.69 d (H9); 4.38 m (H8); 3.88 d, 3.48 dd (H3); C. 3.21 m, C. 2.08 m (H5); 2.81 q (H($HCCH_3$)); 2.59 q, 2.08 m (H6); 1.43 s ($CH_3(CH_3COHCO)$); 1.35 s ($CH_3(CH_3COH)$); 1.21 d (C-$H_3(CH_3CH)$).

Oligomerization Reactions. NMR studies were carried out directly in a 5-mm NMR tube. A solution of dehydroretronecine

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Table I. Rate Constants from Equation 3 for the Alkylation by Macrocyclic Alkaloid Pyrroles at 20 °C of 4-(*p*-Nitrobenzyl)pyridine

pyrrole	K_1, min^{-1}	K_2, min^{-1}	r^2	std dev of regression
monocrotaline (**5**)	0.23 ± 0.0085	0.0013 ± 0.00014	0.999	0.0129
seneciphylline (**6**)	0.59 ± 0.036	0.0010 ± 0.0002	0.998	0.0110
senecionine (**7**)	1.10 ± 0.287	0.0042 ± 0.002	0.955	0.0078
retrorsine (**8**)	1.25 ± 0.087	*a*	0.9798	0.0263
jacozine (**9**)	3.90 ± 0.224	0.0011 ± 0.002	0.994	0.0164
jacobine (**10**)	7.10 ± 0.617	*a*	0.930	0.0564

[a] Data not taken sufficiently long in time to get K_2, K_1 in this case from monoexponential eq 2.

Table II. Fit of Kinetic Data for Monocrotaline Pyrrole (5) to Equation 3 as Shown in Curves in Figure 1

time min	obsd absorb.	fitted $K_1 + K_2$	fast reacn K_1	slow reacn K_2
0.3	0.049	0.0399	0.0397	0.000 14
0.5	0.080	0.0729	0.0726	0.000 26
0.9	0.101	0.1192	0.1187	0.000 45
1.0	0.138	0.1379	0.1374	0.000 53
1.0	0.137	0.1379	0.1374	0.000 53
1.5	0.194	0.1959	0.1951	0.000 79
2.0	0.243	0.2477	0.2466	0.001 06
3.0	0.342	0.3352	0.3336	0.001 59
4.0	0.403	0.4049	0.4027	0.002 12
5.0	0.470	0.4604	0.4577	0.002 65
7.0	0.547	0.5400	0.5363	0.003 70
10.0	0.600	0.6037	0.6035	0.005 28
15.0	0.651	0.6578	0.6499	0.007 90
20.0	0.660	0.6751	0.6646	0.010 49
30.0	0.667	0.6865	0.6709	0.015 64
38.0	0.679	0.6911	0.6714	0.019 71
60.0	0.721	0.7022	0.6715	0.030 68
60.0	0.719	0.7022	0.6715	0.030 68
120.0	0.752	0.7305	0.6716	0.059 00
840.0	0.962	0.9407	0.6716	0.269 21
960.0	0.958	0.9600	0.6716	0.288 53
960.0	0.951	0.9600	0.6716	0.288 53
960.0	0.948	0.9600	0.6716	0.288 53
1440.0	1.000	1.0127	0.6716	0.341 24
1440.0	1.016	1.0127	0.6716	0.341 24
1440.0	1.001	1.0127	0.6716	0.341 24
2160.0	1.054	1.0491	0.6716	0.377 61
2160.0	1.062	1.0491	0.6716	0.377 61

in deuteriochloroform was prepared and an NMR spectrum recorded. A few drops of deuterioacetic acid was added to the solution in the NMR tube and spectra were recorded after 5, 15, and 30 min and 6, 19, and 24 h in one experiment and after 4, 12, 20, and 26 h in another experiment.

Fast atom bombardment mass spectra were recorded of dehydroretronecine in magic bullet, i.e., a 5:1 mixture of dithiothreitol and dithioerythritol as liquid matrix.

Kinetic Studies. Kinetic studies were carried out using 4-(*p*-nitrobenzyl)pyridine (**11**) as nucleophile in large excess (230×) in alkylation reactions of the pyrrolizidine alkaloid pyrroles as previously described.[12] The reactions were carried out at 20 and 30 °C with color formation detected at 565 nm. Analyses of the kinetic data were carried out on the NIH/Prophet computer system Fit Function.[21]

Results and Discussion

In initial studies, the reactions of 4-(*p*-nitrobenzyl)-pyridine (**11**) with simple di- and monoesters of dehydroretronecine and some pyrrolizidine alkaloid macrocyclic diester pyrroles under pseudo-first-order reaction conditions were observed to fit mono- or biexponential rate expressions.[12b]. Since the absorbance at 565 nm was followed for the buildup of product (C) with time (t), a simple chemical reaction (eq 1) was expected to fit the monoexponential eq 2 with C_∞ equal to the final absorbance at reaction completion.[22]

$$A \xrightarrow{K_1} C \tag{1}$$

$$C = C_\infty - C_\infty e^{-K_1 t} \tag{2}$$

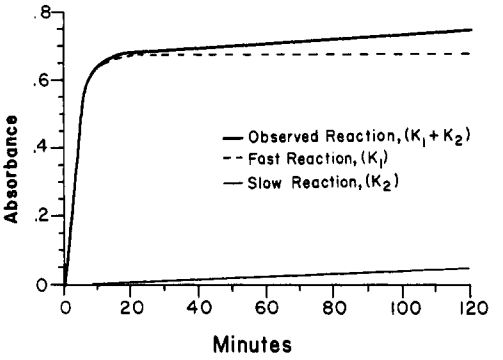

Figure 1. The reaction of monocrotaline pyrrole (**5**) with 4-(*p*-nitrobenzyl)pyridine (**11**) at 20 °C.

Faster reacting compounds, i.e., those that appeared to come to reaction completion in 3–5 min, fit this model. Most compounds, however, when allowed to react longer fit the biexponential expression in eq 3, which corresponds to a parallel reaction (eq 4) where product buildup as absorbance (C) is followed as a function of time (t). The final absorbance at reaction completion for eq 3 is C, which is also equal to $A_0 + B_0$ for the parallel reaction (eq 4).[22b]

$$C = C_\infty - A_0 e^{-K_1 t} - B_0 e^{-K_2 t} \tag{3}$$

$$A \xrightarrow{K_1} C \qquad B \xrightarrow{K_2} C \tag{4}$$

Equation 3 is the general form of the biexponential equation reduced to its simplest form. Other reaction schemes representing various reversible or consecutive reactons can fit biexponential equations that are more complex in terms of K_1, K_2, and t.[22a] However, unrealistic rate constants such as negative values for K_2 indicated that these models were not appropriate.

In an attempt to slow the expected reactions of the macrocyclic diester pyrroles **5–10**, kinetic studies were carried out at 20 °C (Table I). Lowering the reaction temperature separated the two parallel reactions. Figure 1 shows the fast (K_1) and slow (K_2) reaction contribution to the observed absorbance for the reaction of monocrotaline pyrrole (**5**). Table II further compares the raw and fitted data for this reaction scheme. Because the fast reaction is mostly over before the slow reaction starts to make a significant contribution to the observed absorbance, the same value for K_1 can be obtained from either eq 2 by using the early data points or with eq 3 by using all data points. In similar fashion, K_2 can also be obtained from either equation by using the appropriate data points. These kinetic results strongly support the concept that color formation results from two independent parallel reactions (eq 4); one reaction occuring relatively fast with

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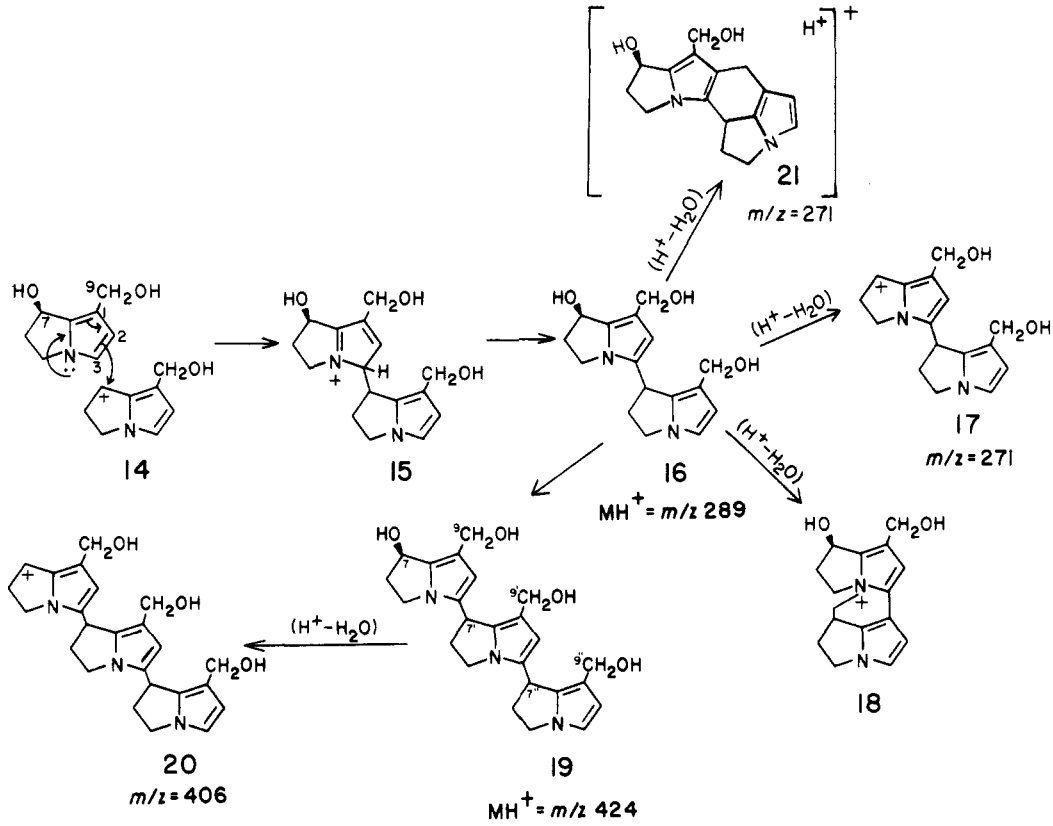
Scheme II



K_1 reflecting the nature of the leaving group on the pyrrole and a second slow reaction where $K_2 \sim 10^{-3}$ min.

A clear trend is observed between K_1 for the macrocyclic pyrroles and the nature of the leaving group at C7. Similarities in K_1 also are observed for seneciphylline pyrrole (**6**), senecionine pyrrole (**7**), and retrorsine pyrrole (**8**) and for jacozine pyrrole (**9**) and jacobine pyrrole (**10**). The epoxide group in proximity to the C7 alkylation site may accelerate the reactions of jacozine pyrrole (**9**) and jacobine pyrrole (**10**) because of concerted decarboxylation of the glycidic acid moiety after cleavage at C7. Monocrotaline pyrrole (**5**), the slowest reacting alkaloid pyrrole, has a simple saturated hydrocarbon system in proximity to the reaction site.

Information on the relative reactivity of C7 and C9 is provided by a comparison (Table III) of dehydrosupinidine (**4**) with dehydroretronecine (**2**) and with 2,3-dihydro-1*H*-pyrrolizin-1-ol (**3**). The relative rate constants (K_1) are not significantly different, which suggests that nucleophilic substitution at C7 and C9 on dehydroretronecine (**2**), and probably pyrrolizidine alkaloid pyrroles, in general, act as bifunctional alkylating agents. Relative rates of substitution at these sites, thus, depend most on the leaving groups present, and some pyrrolizidine alkaloid pyrroles with better leaving groups at C9, such as monocrotaline, may react preferentially at that site.

An explanation of the biexponential fit of the kinetic data for compounds **2** and **5–10** is possible if one considers that pyrrolizidine alkaloid pyrroles are particularly unstable under acidic conditions,[14] giving intensely red or brown solutions that have commonly been ascribed to a polymerization reaction. Hydrolysis of the ester groups and dimerization of the dehydroretronecine nucleus (Scheme II) can occur leading ultimately to oligomeric products. The trimer (**19**), for example, has one pyrrole C7 hydroxyl and three C9 hydroxyls. While disubstitution of the monomer **2** by 4-(*p*-nitrobenzyl)pyridine (**11**) seems unlikely because C7 and C9 are too close to simultaneously accomodate positive charges produced by formation of 4-(*p*-nitrobenzyl)pyridinium ions at these sites (Scheme I), the monomeric pyrrole units of oligomers are not conjugated through interpyrrole linkages and the increased distance between possible reaction sites makes accommodation of a second 4-(*p*-nitrobenzyl)pyridinium ion more likely. Thus color formation as measured at 565 nm, after the reaction is quenched with base, has two independent parallel contributions in a first (K_1) and second (K_2) substitution on the pyrrole oligomer. Each substitution leads to the same chromophore which contributes equally and independently to the total observed absorbance (C) as expressed in eq 4. The color-forming reaction represented by K_2 may not be rate-determining but instead is a constant in the events following a rate-determining oligomerization reaction. The observed kinetics thus would fit eq 3.

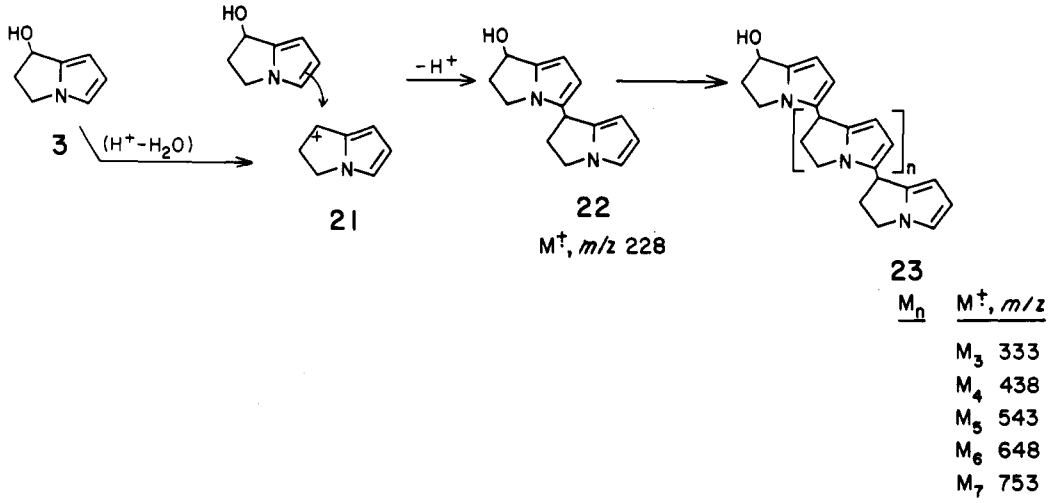
Evidence in support of independent self-reaction of dehydroretronecine (**2**) in acid (Scheme II) has been obtained from NMR and FAB experiments. An 80-MHz ^{1}H NMR spectrum of the reaction kinetics solution with excess 4-(*p*-nitrobenzyl)pyrine obscured most of the resonance region of the dehydroretronecine. The region between 5 and 7 ppm was free of interferences and showed complete loss of the H7 signal, diminished intensity of the H3 and H2 signals, and the appearance of new complex signals. It was difficult to derive much useful information from this study. When ^{1}H NMR (400 MHz) spectra are recorded for a solution of dehydroretronecine (**2**) in the presence of deuterioacetic acid, all of the NMR signals are affected. The H3 (δ 6.54) and H2 (δ 6.16) signals diminish in intensity, and new ones appear further downfield for H3 (δ 6.63) and H2 (δ 6.21) initially. With time these signals increase in intensity and complexity over a 24-h reaction period. The H5 (δ 4.13 m, 3.89 m) signal is more complex, but the center of resonance is about the same.

Table III. Reaction of Necine Base Pyrroles with 4-(*p*-Nitrobenzyl)pyridine at 30 °C

pyrrole	K_1, min^{-1}	K_2, min^{-1}	r^2	std dev
dehydroretronecine (2)	0.33 ± 0.061[a]	0.005 ± 9003[a]	0.998	0.011
2,3-dihydro-1*H*-pyrrolizin-1-ol (3)	0.30 ± 0.015		0.978	0.0052
dehydrosupinidine (4)	0.12 ± 0.001[b]		0.999	0.0230

[a] From eq 3. [b] From eq 2.

Scheme III



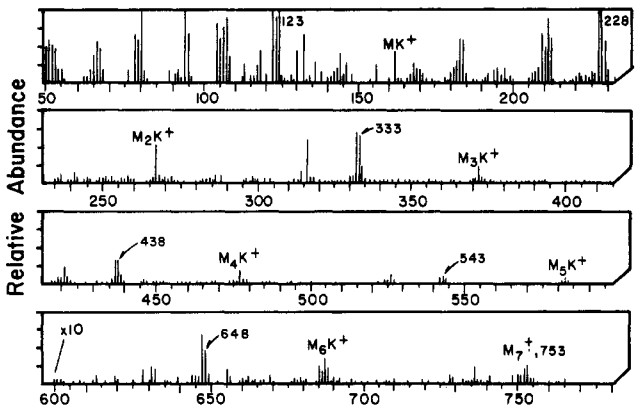


Figure 2. FAB-MS of 2,3-dihydro-1*H*-pyrrolizin-1-ol (3), in 5:1 dithiothreitol–dithioerythritol (magic bullet) matrix with a trace of added potassium chloride.

New multiplets appear for H6 (δ 2.75 m, 2.55), but the ratio of integrated intensities for all H5 and H6 resonances remains constant. The ratio of H3/H5 signal intensities, however, diminishes with time, and the reduction rate depends on the acid concentration. Moreover, the H7 (δ 5.24 q) signal diminishes, and new upfield signals (δ 5.05 q) and (δ 5.13 q) of about equal intensity appear and increase with time. These two quartets are coupled to the new H6 (δ 2.75 m) multiplet as observed in the COSY spectrum. These data support the view that reaction occurs between C7 of one molecule with C3 of another. The upfield shift of H7 is consistent with a change of substitution from OH to olefinic carbon. The appearance of two H7 quartets of approximately equal intensity is consistent with formation of two epimers at C7 through an intermediate carbonium ion. Electrophilic substitution at C3 of dehydroretronecine (2) is also the proposed product-forming step in the colorometric Ehrlich's analytical procedure using *p*-(dimethylamino)benzaldehyde.[23]

The scheme, however, is somewhat more complex. Most important, the simple pair of doublets for H9 (δ 4.68 d, 4.76 d) becomes very complex. The C9 position itself also may undergo substitution. This should be expected since the relative rates of reaction with nucleophile for 2,3-dihydro-1*H*-pyrrolizin-1-ol (3) and dehydrosupinidine (4) are 2.5:1.0 (Table III), and dehydroretronecine C9 must be similarly competetive with C7. Intramolecular cyclization to give a structure such as 21 (Scheme II) or its opposite, i.e., C9–C3 and C7–C2 bonding, is supported by the NMR data which show that the H2/H5 ratio also diminishes with time, although not as rapidly as does the H3/H5 ^{1}H NMR intensity. This possibility also is supported by the FAB spectrum showing an intense *m/e* 271 ion.[24] If intramolecular cyclization with C9–C3 and C7–C2 bond formation occurs a conjugated system results which should absorb at longer wavelengths and possibly contribute to optical density recorded in the kinetics experiments. Additional downfield signals in the spectrum suggest reaction of C7 and C9 with nucleophiles in solution; perhaps deuterioacetate.

Gas-phase decomposition of FAB desorbed ions are known to mimic reactions in solution,[2,4] and this is observed for dehydroretronecine (2). In the FAB spectrum of dehydroretronecine (2) a major peak appears at *m/z* 289 and a much less intense one at *m/z* 424. These are attributed to protonated dimer (16) and trimer (19) (Scheme II); the proton being provided by the matrix glycerol. In addition, there is a major peak at *m/z* 271 (17) and a very small one at *m/z* 406 (20), which result from elimination of water from the dimer and trimer MH^+ ions to give resonance stabilized secondary carbonium ions at C7 or cyclization products of type 18 or type 21. Thus, the most important reaction that appears to take place for dehydroretronecine by itself, under slightly acidic conditions, is dimerization to produce a product of type 16 which undergoes further reaction giving products 18 or 21.

Both dehydrosupinidine (4) and 2,3-dihydro-1*H*-pyrrolizin-1-ol (3) exhibited only simple first-order kinetics when reacted with excess 4-(*p*-nitrobenzyl)pyridine (11) (Table III). Even though these monohydroxyl pyrroles also undergo polymerization reactions under acid conditions,

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(24) We thank a referee for pointing this possibility out to us.

the oligomers formed are monofunctional to nucleophilic attack by 4-(*p*-nitrobenzyl)pyridine (Scheme III). The observed kinetics are then consistent with eq 1 and 2 for a simple pseudo-first-order reaction. Evidence for oligomer formation under acid conditions is evident in the FAB spectrum of 2,3-dihydro-1*H*-pyrrolizin-1-ol (**3**), which shows peaks for oligomer ions up to a heptamer (M_7, Figure 2). This compound (**3**) seems to undergo oligomerization much more readily than does dehydroretronecine (**2**). Each oligomer displays a series of ion peaks at $(M - H)^+$, $M^{\bullet+}$, MH^+, $[MH - H_2O]^+$, and MK^+ (when a potassium salt is added to the FAB matrix). Similar aromatic nitrogen compounds from fossil fuels have been shown to give characteristic $(M - H)^+$, $M^{\bullet+}$, and MH^+ clusters under FAB conditions.[25] The facile loss of water from the allylic hydroxyl of each oligomer MH^+ gives rise to the abundant $[MH - H_2O]^+$ ions. It is this facile loss of water under acid-catalyzed conditions, whether under FAB experimental conditions or in solution such as the conditions of kinetic experiments that lead to a resonance stabilized allylic carbonium ion (**21**, Scheme III) which can readily undergo nucleophilic attack by a neutral pyrrole molecule, monomer **3**, or oligomer **22** or **23**. Because the allylic hydroxyl group of the oligomers **22** and **23** is also acid labile, polymerization most likely occurs on each end of the forming oligomer chain **23**. It is expected that dehydrosupinidine (**4**) polymerizes in similar fashion.

Dehydroretronecine (**2**) and the macrocyclic pyrrolizidine alkaloid pyrroles **5**–**10** have the potential for being bifunctional alkylating agents. Both in vitro and in vivo evidence has been published showing that monocrotaline pyrrole may induce DNA cross-linking, presumably by bialkylation,[26] and recently direct spectroscopic evidence has been obtained for cross-linking of strands in the duplex by mitomycin C, a compound with structural similarities to dehydroretronecine, and its derivatives.[27] Our results indicate that these bifunctional monomeric pyrrolizidine derived pyrroles, in fact, can undergo polymerization to produce multifunctional oligomers. This may be significant when one considers alkylation and repair mechanisms of DNA. Oligomers of dehydroretronecine (Scheme II) are polyhydroxylic compounds that would exhibit adsorption properties toward other polar molecules because of the multiple hydrogen bond adsorption sites. Molecular orientations in solution due to such interactions could place these pyrrole polymers in a more facile possition to alkylate DNA chains. The polyhydroxylic nature of these oligomers may also help explain the relatively rapid demise of enzymes used to digest DNA that has been reacted with dehydroretronecine (**2**) and the inability to completely hydrolyze such DNA adducts.[9]

Acknowledgment. This work was supported by Grants from the National Institutes of Health (ES-03874 and ES-00210). This is Technical Paper 7890 from the Oregon Agricultural Experiment Station. We thank Roger Kohnert for obtaining the proton NMR spectra. The multinuclear Bruker AM 400 NMR spectrometer was purchased in part through grants from the National Science Foundation (CHE 8216190) and from the M.J. Murdock Charitable Trust to Oregon State University.

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Total Synthesis of Linear Polyprenoids. 2.[1] Improved Preparation of the Aromatic Nucleus of Ubiquinone

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Received January 15, 1987

Highly efficient copper-catalyzed polymethoxylation of tribromocresol is the key process in a three-step, practical approach to obtain ubiquinone 0 from *p*-cresol. Short syntheses of several ubiquinones were achieved via direct, copper-mediated coupling of 2-lithio-3,4,5,6-tetramethoxytoluene to the appropriate polyprenyl bromide.

Introduction

Quinones and hydroquinones with polyprenyl side chains, such as ubiquinones, plastoquinones, phylloquinone (vitamin K_1), and menaquinones (vitamin K_2), are widely distributed in plant and animal tissues.[2] In addition to vital roles in promoting electron transfer in respiratory chains and photosynthesis, these compounds also exhibit various pharmacological activities.[3] Of special interest is ubiquinone 10 (coenzyme Q_{10}), **1**, which is used clinically as a cardiovascular agent and has attracted significant synthetic activity over the past two decades.[4] However, because construction of linear polyprenoid chains still represents a major synthetic challenge, the practical total

† Incumbent of the Joseph and Madeleine Nash Career Development Chair established by Fondacion Madelon, Zurich, Switzerland.

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0022-3263/87/1952-3872$01.50/0 © 1987 American Chemical Society