of benzoyl-CoA was compared with the ^{1}H NMR of other acyl-CoA derivatives; the chemical shift of the phenyl group appeared at δ 7.6–7.9 ppm (m, 5 H, C_6H_5).

Synthesis of Citric Acid Using Acetyl-CoA Recycling (Scheme II). A typical reaction was carried out as follows: Oxalacetic acid (500 mg, 3.8 mmol) was dissolved and neutralized with 6 mL of 2 M Tris base to pH 7.8. To start the reaction and every 2 or 3 h, 50-mg portions of *S*-acetylthiocholine iodide (500 mg, 1.7 mmol) and 200-μL aliquots of oxalacetate solution were added to CoA (1 mg, 1.3 μmol) and the enzyme citrate synthase (EC 4.1.3.7) (1000 units). The mixture was left to react in a shaker incubator at 40 °C. The progress of the reaction was monitored by detecting the formation of citric acid using ^{1}H NMR. After 3 days, the amount of citrate formed was determined by ^{1}H NMR using ethanol as an internal standard. Citric acid was purified from the reaction mixture by acidifying the mixture to pH 1 and then lyophilizing. The resulting white powder was extracted with methanol–acetone (1:50). Solvents were removed under reduced pressure to provide an oil. Citric acid was determined by ^{1}H NMR. The amount of citric acid was determined by using absolute ethanol as the internal standard. The total turnover number of 1160 was obtained for acetylcoenzyme A. Yield 88% based on acetylthiocholine or 38% based on oxalacetic acid.

Acetyl-CoA Recycling Using Immobilized Enzymes. The procedure was repeated using citrate synthase (1000 units) immobilized on glass beads.[14] At the end of the reaction, the immobilized enzyme was removed by filtration. The enzyme was assayed after adding the substrates oxalacetic acid and acetyl-CoA using HPLC to monitor the formation of CoA.

Synthesis of L-Acetylcarnitine Using Acetyl-CoA Recycling (Scheme III). DL-Carnitine (1 g, 5 mmol) dissolved in distilled water and neutralized with 2 M K_3PO_4 to pH 7.8 was added to CoA (1 mg, 1.3×10^{-3} mmol). The enzyme carnitine acetyltransferase (EC 2.3.1.7) (500 units) was added in 60-unit aliquots, and *S*-acetylthiocholine (500 mg, 1.7 mmol) was added in 50-mg portions to the reaction mixture every 2 or 3 h. The mixture was left to react in a shaker incubator at 40 °C for 3 days. The formation of L-acetylcarnitine was monitored using either 300-MHz ^{1}H NMR or HPLC with reverse-phase C-18 column and an UV detector at 208 nm. The amount of L-acetylcarnitine formed was determined by ^{1}H NMR using ethanol as an internal standard and corresponded to a recycling number of 340 for acetyl-CoA, corresponding to a 26% yield based on acetyl thiocholine or 18% based on L-carnitine in the starting racemate. The L-acetylcarnitine was purified by HPLC, on a reverse-phase preparative C-18 column using 0.1 M phosphate buffer, pH 5.5, as mobile phase and detected at 208 nm. L-Acetylcarnitine was confirmed by ^{1}H NMR and its optical purity determined by using the chiral shift reagent, tris[3-((trifluoromethyl)hydroxymethylene)-*d*-camphorato]europium(III).[20] When an equimolar quantity of the chiral shift reagent was added to DL-acetylcarnitine, resolution of the two enantiomers was observed on the ^{1}H NMR. The acetyl group appears as two singlets at 2.04 and 2.05 ppm and the trimethylammonium group as two singlets at 3.5 and 3.6 ppm. Addition of the chiral shift reagent to the acetylcarnitine purified from the above reaction showed only one isomer in the ^{1}H NMR. To verify, 5 mg of DL-acetylcarnitine was added to the NMR tube producing a small new peak at 2.04 ppm belonging to D-acetylcarnitine.

Acknowledgment. Partial support for this work was provided by Grant GM35327 of the National Institute of General Medical Sciences.

Registry No. **1**, 108-02-1; **2b**, 24395-12-8; **2c**, 63512-62-9; **3a**, 1866-15-5; **3d**, 10561-14-5; CoA, 85-61-0; acetyl-CoA, 72-89-9; propionyl-CoA, 317-66-8; butyryl-CoA, 2140-48-9; benzoyl-CoA, 6756-74-7; $(PhCO)_2O$, 93-97-0; $(HO_2CCH_2)_2C(OH)CO_2H$, 77-92-9; $HO_2CCOCH_2CO_2H$, 144-62-7; citrate synthase, 9027-96-7; L-acetylcarnitine, 3040-38-8; carnitine acetyltransferase, 9029-90-7; DL-carnitine, 406-76-8.

Supplementary Material Available: ^{1}H NMR spectrum of *S*-benzoylthiocholine iodide **3d** (1 page). Ordering information is given on any current masthead page.

Strained Heterocyclic Systems. 20.[1] Basicities of Bicyclic Quinoxalines

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Received January 7, 1991

The influence of ring strain effects on the basicity of quinolines was first reported in 1967.[3] The initial studies were extended to quinoxalines[4] and, more recently, to 1-azatriptycene.[5] In this report a similar correlation is applied to a series of bicyclic quinoxalines.

Strain effects in bicyclic alkanes are well known[6] and are readily reflected in an NMR parameter such as the $J(^{13}C–H)$ value for bridgehead protons. For instance, the one-bond coupling constants for that position in the closely related series bicyclo[2.2.2]octane, bicyclo[2.2.1]heptane, and bicyclo[2.1.1]hexane are 134.3, 140.1, and 150.5 Hz, respectively,[7] reflecting the increased s character in the C–H bond due to orbital rehybridization.[8] For comparison, the same parameter for cyclopentane is 128 Hz.[9]

With this in mind, the following compounds were chosen for study: 2,3-dihydro-1*H*-cyclopenta[*b*]quinoxaline (**1**), 1,2,3,4-tetrahydro-1,4-ethanophenazine (**2**), 1,2,3,4-tetrahydro-1,4-methanophenazine (**3**), and 2,3-dihydro-1,3-methano-1*H*-cyclopenta[*b*]quinoxaline (**4**). Compounds **1–3** were prepared by literature methods; a preliminary account of **4** has been reported.[10] The pK_a values of the conjugate acids were determined by spectrophotometric titration, and the results in order of decreasing basicity are summarized in Table I, along with values for model compounds 2,3-dimethylquinoxaline (**5**) and quinoxaline (**6**).

Compounds **1–4** were all less basic than **5** and more basic than **6**. The latter fact was somewhat unexpected, although strain effects in ortho-annelated quinoxalines were previously observed to be more compressed than in analogous quinolines.[4,13] The basicities of **3** and **4** were essentially the same, and both compounds were the least basic of the series studied. Such order was consistent with

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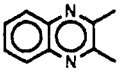
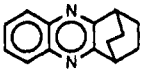
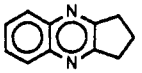
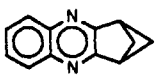
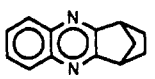
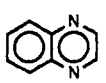
Table I. Basicities of Selected Quinoxalines

compd	structure	λ (nm)	pK_a	ref
5			2.08	11
2		337.0 341.0 344.6	1.51 ● 0.07	*a*
1		346.0 351.0 354.6	1.38 ● 0.07	*a*
4		332.0 336.0 339.6	1.16 ± 0.02	*a*
3		342.0 346.0 349.6	1.12 ± 0.06	*a*
6			1.03	12

[a] Present work.

increased s character of the hybrid orbital containing the electron pair on the nitrogen atom.[3]

Experimental Section

General. Melting points were determined on a modified Hershberg apparatus with matched Anschutz thermometers. NMR spectra were obtained on a Brucker AC 200 spectrometer; chemical shifts are reported in ppm (δ) and proton assignments in 4 are labeled a or s for anti and syn, respectively. GC/MS analyses were performed on Hewlett-Packard 5890II/5971A instruments (EI-MS 70 eV). Elemental analyses were determined on a Carlo Erba Strumentatione Analyzer, Model 1106. Bicyclic α-diketones were prepared from the corresponding alkenes via cis-dihydroxylation[14] and Swern oxidation.[15] Quinoxalines **1**, **2**, **3**, and **5** were prepared by reported procedures and chromatographed on neutral alumina (activity I) with elution by chloroform. Commercial quinoxaline (**6**) was vacuum sublimed immediately before use.

2,3-Dihydro-1*H*-cyclopenta[*b*]quinoxaline (1): mp 99.0–99.5 °C (lit.[16] mp 99.2–99.7 °C); MS *m/z* (relaive abundance) 170 (M^+, 100), 169 (85).

1,2,3,4-Tetrahydro-1,4-ethanophenazine (2): mp 136–137 °C (lit.[17] mp 138–139 °C); MS *m/z* 210 (M^+, 100), 209 (58), 182 (64), 181 (90).

1,2,3,4-Tetrahydro-1,4-methanophenazine (3): mp 106.8–107.2 °C (lit.[17] 110–111 °C); MS *m/z* 196 (M^+, 75), 195 (51), 168 (100).

2,3-Dihydro-1,3-methano-1*H*-cyclopenta[*b*]quinoxaline (4). To a stirred solution of bicyclo[2.1.1]hexane-2,3-dione[10] (225 mg, 2.04 mmol) in diethyl ether (10 mL) at 20 °C under nitrogen was added dropwise by syringe a solution of *o*-phenylenediamine (220 mg, 2.04 mmol) in dichloromethane (4 mL). The reaction mixture was stirred at 20 °C for 14 h, concentrated at reduced pressure, diluted with water (10 mL), and extracted three times with 5-mL portions of dichloromethane. The combined extracts were dried ($MgSO_4$) and concentrated at reduced pressure to give a yellow semisolid, the solution of which in *tert*-butyl methyl ether was filtered through alumina (2 g, activity III) and concentrated at reduced pressure to give a residual solid. The crude product was recrystallized from diethyl ether (2 mL; overnight at −25 °C) to give 101 mg (27%) of colorless, crystalline **4**: mp 97–99 °C. The yield was greatly enhanced by concentrating the mother liquor at reduced pressure and distilling the residue in a short-path apparatus (70 °C bath, 0.001 Torr) whereby **4** crystallized immediately in the cold receiver (−70 °C): MS *m/z* 182 (M^+, 84), 181 (100); ^1H NMR ($DCCl_3$) δ 2.53 (AA′ part of an AA′XX′ spectrum, $J_{2a,2s}$ = −6.8 Hz, $J_{2s,10s}$ = +0.4 Hz, $J_{2a,10a}$ = −10.0 Hz, $J_{2a,10s}$ = −0.3 Hz, H-2s), 3.03 (m, H-2a), 3.47 (t, $J_{1,2a}$ = 2.9 Hz, H-1), 7.60 and 7.93 (AA′XX′ spectrum, $J_{5,6}$ = +8.2 Hz, $J_{5,7}$ = +1.5 Hz, $J_{5,8}$ = +0.4 Hz, $J_{6,7}$ = +7.1 Hz, H-5 and H-6); ^{13}C NMR ($DCCl_3$) δ 46.7 (ddqui, $J_{C\text{-}1,H\text{-}1}$ = 161.2 Hz, $J_{C\text{-}1,H\text{-}3}$ = 9.4 Hz, $J_{C\text{-}1,H\text{-}2}$ = 2.3 Hz, C-1), 55.8[18] (dddd, $J_{C\text{-}2,H\text{-}2}$ = 146.2 and 143.3 Hz, $J_{C\text{-}2,H\text{-}10s}$ = 12.9 Hz, $J_{C\text{-}2,H\text{-}10a}$ = 4.6 Hz, C-2), 128.0 (dd, $J_{C\text{-}6,H\text{-}6}$ = 162.5 Hz, $J_{C\text{-}6,H\text{-}8}$ = 8.9 Hz, C-6), 128.5 (ddm, $J_{C\text{-}5,H\text{-}5}$ = 165.3 Hz, $J_{C\text{-}5,H\text{-}7}$ = ca. 6 Hz, C-5), 140.1 (s, C-4a), 168.5 (s, C-3a). Anal. Calcd for $C_{12}H_{10}N_2$: C, 79.10; H, 5.53; N, 15.37. Found: C, 79.23; H, 5.34; N, 15.45.

2,3-Dimethylquinoxaline (5): mp 105.5–106.0 °C (lit.[19] mp 106 °C); MS *m/z* 158 (M^+, 95), 117 (100), 76 (29).

Quinoxaline (6): mp 31.8–32.4 °C (lit.[20] mp 29–30 °C); MS *m/z* 130 (M^+, 100), 103 (46), 76 (39).

Basicity Determinations. Ultraviolet spectra were recorded on a Cary 219 spectrophotometer in 1-cm cells in a water-jacketed chamber maintained at 25.0 °C by a Lauda K-2/R bath. The procedure has been described elsewhere.[21] Measurements were made at three wavelengths, using five solutions of different pH values (0.3–2.00) at each wavelength. The appropriate buffer was used as a blank for each determination. The data are corrected for ionic strength effects.

Acknowledgment. This work was funded by a faculty research grant from Williams College and by the Deutsche Forschungsgemeinschaft. A.K. thanks the Stiftung Stipendien-Fonds for a fellowship (1987–1988). Generous support from Dr. Peter M. Wege II provided summer stipends for N.I.L. and J.R.C.

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