of benzoyl-CoA was compared with the $^1H$ NMR spectrum of other acyl-CoA derivatives; the chemical shift of the phenyl group appeared at $\delta$ 7.8-7.9 ppm ($\delta \ H$, $\delta $ H$_2$C$_6$H$_5$).

Synthesis of Citric Acid Using Acetyl-CoA Recycling (Scheme II). A typical reaction was carried out as follows: Oxalacetate (600 mg, 5.1 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-$\mu$L aliquots of oxalacetate solution were added to CoA (1 mg, 1.3 mmol) and the enzyme citrate synthase (EC 5.3.3.2) (1000 unita). 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 $^1H$ NMR. After 3 days, the amount of citrate formed was determined by $^1H$ 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-acetones (1:50). Solvents were removed under reduced pressure to provide an oil. Citric acid was determined by $^1H$ NMR.

The amount of citric acid formed was determined by using absolute ethanol as the internal standard. The total turnover number of 1160 was obtained for oxaloacetem A. Yield 88% based on oxaloacetem or 38% based on oxalacetem acid.

The Acetyl-CoA Recycling Using Immobilized Enzymes. The procedure was repeated using citrate synthase (100 units) immobilized on glass beads. At the end of the reaction, the immobilized enzyme was removed by filtration. The enzyme was assayed after adding the substrates oxalacetem acid and acetyl-CoA using the formaldehyde [CoA] recycle.

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$_2$PO$_4$ to pH 7.8 was added to CoA (1 mg, 1.3 $\times$ 10$^3$ mmol). The enzyme carnitine acetyltransferase (EC 2.3.1.7) (500 units) was added in 80-$\mu$L aliquots, and S-acetylthiocholine iodide (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 $^1H$ NMR or HPLC with reverse-phase C18 column and an UV detector at 208 nm. The amount of L-acetylcarnitine formed was determined by $^1H$ 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 C18 column using 0.1 M phosphate buffer, pH 5.5, as mobile phase and detected at 208 nm. L-Acetylcarnitine was confirmed by $^1H$ NMR and its optical purity determined by using the chiral shift reagent, tria[3-(trifluoromethyl)hydroxy-methylen]-d-camphoro][europium(III)]. After an equilibrium quantity of the chiral shift reagent was added to L-acetylcarnitine, resolution of the two enantiomers was observed on the $^1H$ NMR. The two acetylcarnitines were detected as 2.04 and 2.06 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 $^1H$ 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.

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Registry No. 1, 108-02-1; 2b, 24305-12-8; 2c, 63512-62-9; 3a, 1966-15-5; 3d, 10611-14-4; CoA, 85-61-0; acetyl-CoA, 72-89-9; propionyl-CoA, 317-68-6; butyryl-CoA, 2140-48-9; benzoyl-CoA, 6768-74-7; P(CHO)O, 85-97-9; HOC(CHO)CH(COH), 77-92-9; HOC(CHOH)CH(COH), 1144-62-7; L-acetylcarnitine, 5007-25-7; L-acetylcarnitine, 3040-38-8; carnitine acetyltransferase, 9029-90-7; DL-carnitine, 406-76-8.

Supplementary Material Available: $^1H$ NMR spectrum of S-benzoylthiocholine iodide is 5 pages. Ordering information is given on any current masthead page.

Strained Heterocyclic Systems. 20.1 Basics of Bicyclic Quinoxalines.

J. Hodge Markgraf,* John R. Cort, Howard A. Davis, Neal I. Lindeman, and Christopher R. Myers

Department of Chemistry, Williams College, Williamstown, Massachusetts 01267

Manfred Christl and Arno Kraft

Institut für Organische Chemie der Universität Am Hubland, D-8700 Würzburg, F.R.G.

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The influence of ring strain effects on the basicity of quinoxalines was first reported in 1967.7 The initial studies were extended to quinoxalines and, more recently, to 1-azatriptycenes.8 In this report a similar correlation is applied to a series of bicyclic quinoxalines.

Strain effects in bicyclic alkyls are well known6 and are readily reflected in an NMR parameter such as the $J(3H-C^6H)$ value for bridgehead protons. For instance, the one-bond coupling constants for that position in the closely related series bicyclo[2.2.1]heptane, bicyclo[3.2.1]octane, and bicyclo[2.1.1]hexane are 134.3, 140.1, and 150.5 Hz, respectively,7 reflecting the increased a character in the C-H bond due to orbital hybridization.8 With this in mind, the following compounds were chosen for study: 2,3-dihydro-1H-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-1H-cyclopenta[b]quinoxaline (4). Compounds 1-3 were prepared by literature methods; a preliminary account of 4 has been reported.9 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 2,5-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 quinoxalines.10,11 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

(2) (a) Based in part on the Honors Thesis of N.L.L., Williams College, 1990. (b) Present address: University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K.
increased a 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 b 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 Strumentazione Analyzer, Model 1106. Bicyclic α,δ-diketones were prepared from the corresponding alkenes via cis-dihydroxylation 14 and Swern oxidation. 16 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-1H-cyclopenta[b]quinoxaline (1): mp 99.0-99.5 °C (lit. 18 mp 99.5 °C); MS m/z 170 (M+, 100), 169 (85) 17, 18.

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). 19

2,3,4-Tetrahydro-1,4-methanophenazine (3): mp 106.5-107.2 °C (lit. 16 110-111 °C); MS m/z 196 (M+, 75), 195 (61), 168 (100). 20