analyses, spectra) were often collected on mesylate preparations other than those referred to in Table I or below. The "times" given for those reactions conducted in acetonitrile under reflux are measured from the moment of addition of the carbonyl compound to the hot HMIB solution until heating was discontinued; the temperature may have dropped a bit below the reflux temperature during the additions. Solvent volumes do not account for the small quantity of solvent that may have been used for rinsing purposes.

[Hydroxy(mesyloxy)iodo]benzene (4) previously reported by the groups of Zefirov[10] and Stang[12] was prepared from (diacetoxyiodo)benzene, $PhI(OAc)_2$, and methanesulfonic acid in acetonitrile. In one particular prep, 51.38 g (ca. 0.16 mol) of the diacetate gave 43.86 g (87%) of HMIB *after* its recrystallization from MeCN; mp 123–126 °C [lit.[10,12] mp 119–120 °C, 120–122 °C].

2-(Mesyloxy)-3-pentanone. To a solution of HMIB (3.16 g, 10.0 mmol) in MeCN (25 mL), stirred and heated under reflux, was added a solution of 3-pentanone (5 mL) in MeCN (5 mL). After 12 min at reflux, the reaction mixture was concentrated to an oil. A solution of the oil in CH_2Cl_2 (50 mL) was washed with H_2O (2 × 25 mL), dried ($MgSO_4$), and concentrated. Flash column chromatography of the residual oil on silica gel (37 g) first with hexanes (160 mL, to remove PhI) and then with CH_2Cl_2 (300 mL) gave 2-(mesyloxy)-3-pentanone as a bright yellow oil: yield 1.57 g (87%); ^1H NMR ($CDCl_3$) δ 1.07 (t, 3 H), 1.53 (d, 3 H), 2.62 (q, 2 H), 3.16 (s, 3 H), 5.07 (q, 1 H); IR (neat) ca. 1725 cm^{-1} (C=O). Anal. Calcd for $C_6H_{12}O_4S$: C, 39.99; H, 6.71. Found: C, 40.22; H, 6.93.

α-(Mesyloxy)cyclohexanone. To a stirred mixture of HMIB (3.16 g, 10.0 mmol) and CH_2Cl_2 (25 mL) at room temperature was added a solution of cyclohexanone (4 mL) in CH_2Cl_2 (4 mL). After 70 min, the reaction mixture, consisting of a solution phase and a floating globule of oil, was diluted with CH_2Cl_2 (15 mL), washed with H_2O (2 × 25 mL), dried ($MgSO_4$), and concentrated. Treatment of the residual oil with Et_2O (5 mL, 1 h, dry ice/acetone bath) gave α-(mesyloxy)cyclohexanone as a white powder: yield 0.949 g (49%); mp ~55 °C. Recrystallization of the crude product (0.821 g) from Et_2O (15 mL) returned 0.662 g, mp 58–59 °C.

In a similar experiment [HMIB (3.18 g, 10.0 mmol), cyclohexanone (1.03 g, 10.0 mmol), 21 h], α-(mesyloxy)cyclohexanone was isolated in 58% yield, mp 59–59.5 °C. ^1H NMR ($CDCl_3$) δ 1.13–2.77 (br m, 8 H), 3.15 (s, 3 H), 4.77–5.27 (m, 1 H); IR (Nujol) ca. 1728 cm^{-1} (C=O). Anal. Calcd for $C_7H_{12}O_4S$: C, 43.74; H, 6.29. Found: C, 43.75; H, 6.43.

3-(Mesyloxy)-2,4-pentanedione. To a stirred mixture of HMIB (3.16 g, 10.0 mmol) and MeCN (25 mL) at room temperature was added a solution of 2,4-pentanedione (1.21 g, 12.1 mmol) in MeCN (5 mL). After 15 min, the clear, colorless solution that resulted was concentrated to a light yellow oil. Flash column chromatography of the oil on silica gel first with hexanes (125 mL, to remove PhI) and then with CH_2Cl_2 (265 mL) gave 3-(mesyloxy)-2,4-pentanedione as a nearly colorless oil: yield 1.17 g (60%); ^1H NMR ($CDCl_3$) δ 2.37 (s, 6 H), 3.28 (s, 3 H), 5.55 (s, 1 H). Anal. Calcd for $C_6H_{10}O_5S$: C, 37.11; H, 5.19. Found: C, 36.85; H, 5.47.

α-Mesyloxylation of 2-Butanone. To a solution of HMIB (3.16 g, 10.0 mmol) in MeCN (25 mL), stirred and heated under reflux, was added 8 mL of 2-butanone. After 27 min at reflux, the reaction mixture was concentrated to an oil. A solution of the oil in CH_2Cl_2 (50 mL) was washed with H_2O (40 mL, an emulsion resulted) and saturated NaCl (aqueous), dried ($MgSO_4$), and concentrated. An ^1H NMR spectrum ($CDCl_3$) of the residual oil was recorded and reveals a fairly clean mixture of iodobenzene and (mesyloxy)butanones. The relative areas of the methyl triplet at δ 1.1 for 1-(mesyloxy)-2-butanone and the methyl doublet at δ 1.6 for 3-(mesyloxy)-2-butanone are consistent with a 1:1.26 mole ratio of these compounds.

α-(Mesyloxy)acetone: chromatographic workup; light-yellow oil (1.16 g); ^1H NMR ($CDCl_3$) δ 2.20 (s, 3 H), 3.16 (s, 3 H), 4.78 (s, 2 H); IR (neat) ca. 1738 cm^{-1} (C=O). Anal. Calcd for $C_4H_8O_4S$: C, 31.57; H, 5.30. Found: C, 31.68; H, 5.31.

α-(Mesyloxy)acetophenone: ether workup; light-brown solid (1.34 g); mp (Et_2O) 76–77 °C; ^1H NMR ($CDCl_3$) δ 3.20 (s, 3 H), 5.42 (s, 2 H), 7.08–8.12 (m, 5 H); IR (Nujol) ca. 1705 cm^{-1} (C=O). Anal. Calcd for $C_9H_{10}O_4S$: C, 50.46; H, 4.70. Found: C, 50.13; H, 4.47.

Cyclopropyl (mesyloxy)methyl ketone: ether workup; pale greenish-yellow powder (1.615 g); mp (Et_2O) 45–46 °C; ^1H NMR ($CDCl_3$) δ 0.87–1.37 (m, 4 H), 1.70–2.33 (m, 1 H), 3.19 (s, 3 H), 4.97 (s, 2 H); IR (Nujol) ca. 1710 cm^{-1} (C=O). Anal. Calcd for $C_6H_{10}O_4S$: C, 40.44; H, 5.66. Found: C, 40.51; H, 5.77.

(Mesyloxy)methyl 2-thienyl ketone: HMIB (1.45 g, 4.59 mmol) recovered; ether workup; white solid (0.86 g); mp (1:1, v/v Et_2O–CH_2Cl_2) 87–88 °C; ^1H NMR (CD_3COCD_3) δ 3.27 (s, 3 H), 5.55 (s, 2 H), 7.28 (symmetrical three-line m, 1 H), 8.05 (symmetrical two-line m, 2 H); IR (Nujol) ca. 1670 cm^{-1} (C=O). Anal. Calcd for $C_7H_8O_4S_2$: C, 38.17; H, 3.66. Found: C, 37.80; H, 3.55.

Dibenzoyl(mesyloxy)methane: ether workup; powder (3.06 g); mp (MeOH, crystalline rosettes) 153–154 °C; ^1H NMR (CD_3SOCD_3) δ 3.44 (s, 3 H), 7.20–7.83 (m, 7 H), 7.83–8.27 (m, 4 H); IR (Nujol) ca. 1688 cm^{-1} (C=O). Anal. Calcd for $C_{16}H_{14}O_5S$: C, 60.37; H, 4.43. Found: C, 60.17; H, 4.53.

2-(Mesyloxy)-5,5-dimethylcyclohexane-1,3-dione: ether workup; white solid (1.89 g); mp (CH_2Cl_2) 147–148 °C; ^1H NMR (CD_3COCD_3) δ 1.14 (s, 6 H), 2.46 (s, 4 H), 3.32 (s, 3 H), 6.56 (s, 1 H); IR (Nujol) broad adsorption, ca. 1540–1600 cm^{-1}. Anal. Calcd for $C_9H_{14}O_5S$: C, 46.14; H, 6.02. Found: C, 46.05; H, 5.91.

Ethyl (mesyloxy)acetoacetate: chromatographic workup; nearly colorless oil (1.81 g); ^1H NMR ($CDCl_3$) δ 1.28 (t, 3 H), 2.33 (s, 3 H), 3.20 (s, 3 H), 4.27 (q, 2 H), 5.38 (s, 1 H), 2.12 (d, impurity, ca. 0.4 H); IR (neat) ca. 1638, 1659 (sh) cm^{-1} (C=O). Anal. Calcd for $C_7H_{12}O_6S$: C, 37.49; H, 5.39. Found: C, 37.62; H, 5.48.

Ethyl (mesyloxy)benzoylacetate: chromatographic workup; light-yellow oil (2.82 g); ^1H NMR ($CDCl_3$) δ 1.16 (t, 3 H), 3.23 (s, 3 H), 4.24 (q, 2 H), 6.23 (s, 1 H), 7.23–8.23 (m, 5 H); IR (neat) ca. 1690 cm^{-1} (ketone C=O), ca. 1749 cm^{-1} (ester C=O). Anal. Calcd for $C_{12}H_{14}O_6S$: C, 50.34; H, 4.93. Found: C, 49.94; H, 5.09.

Diethyl (mesyloxy)malonate: chromatographic workup; bright-yellow oil (1.64 g); ^1H NMR ($CDCl_3$) δ 1.32 (t, 6 H), 3.24 (s, 3 H), 4.31 (q, 4 H), 5.43 (s, 1 H); IR (neat) ca. 1748 cm^{-1} (C=O). Anal. Calcd for $C_8H_{14}O_7S$: C, 37.79; H, 5.55. Found: C, 37.86; H, 5.57.

Registry No. 4, 105551-42-6; H_3CCOCH_3, 67-64-1; H_3CCOCH_2OMs, 23479-35-8; EtCOEt, 96-22-0; $EtCOCH(OMs)CH_3$, 111772-76-0; $PhCOCH_3$, 98-86-2; $PhCOCH_2OMs$, 20187-61-5; $CH_2(COCH_3)_2$, 123-54-6; $MsOCH(COCH_3)_2$, 111793-44-3; $CH_2(COPh)_2$, 120-46-7; $MsOCH(COPh)_2$, 111772-79-3; $H_3CCOCH_2CO_2Et$, 141-97-9; $H_3CCOCH(OMs)CO_2Et$, 111772-81-7; $PhCOCH_2CO_2Et$, 94-02-0; $PhCOCH(OMs)CO_2Et$, 111772-82-8; $CH_2(CO_2Et)_2$, 105-53-3; $MsOCH(CO_2Et)_2$, 88973-33-5; $PhI(OAc)_2$, 3240-34-4; H_3CSO_3H, 75-75-2; H_3CCOEt, 78-92-2; $MsOCH_2COEt$, 102096-20-8; $H_3CCOCH(OMs)CH_3$, 77611-73-5; cyclopropyl methyl ketone, 765-43-5; cyclopropyl (mesyloxy)methyl ketone, 111772-77-1; methyl 2-thienyl ketone, 88-15-3; (mesyloxy)methyl 2-thienyl ketone, 111772-78-2; cyclohexanone, 108-94-1; 2-(mesyloxy)cyclohexanone, 20187-64-8; 5,5-dimethylcyclohexane-1,3-dione, 126-81-8; 5,5-dimethyl-2-(mesyloxy)cyclohexane-1,3-dione, 111772-80-6.

(12) Stang, P. J.; Surber, B. W.; Chen, Z.-C.; Roberts, K. A.; Anderson, A. G. *J. Am. Chem. Soc.* **1987**, *109*, 228.

Studies on Polycyclic Azaarenes. 2.[1] Synthesis of *trans*-3,4-Dihydroxy-3,4-dihydrobenz[*c*]acridine and *trans*-8,9-Dihydroxy-8,9-dihydrobenz[*c*]acridine

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The benz[*c*]acridine derivatives are found to be carcinogenic in nature.[2] In analogy with corresponding

(1) Part 1: Ray, J. K.; Kar, G. K.; Chatterjee, B. G. *Tetrahedron* **1984**, *40*, 2959.

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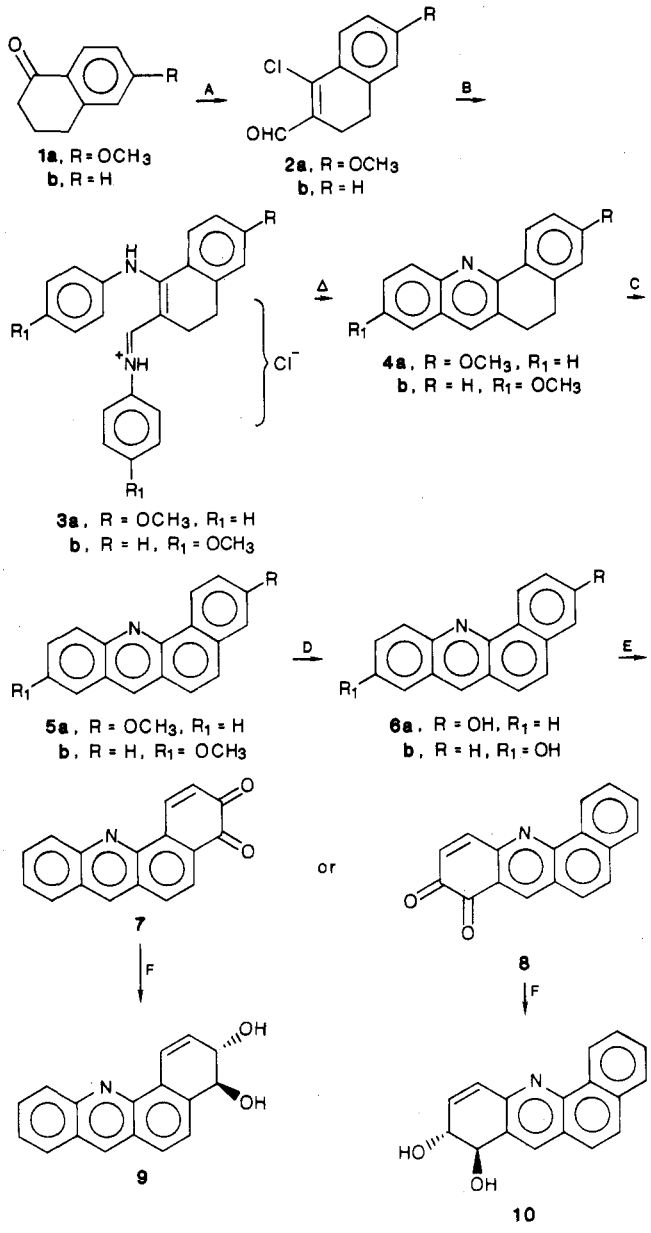
Scheme I[a]



[a] (A) $POCl_3$ + DMF; (B) aniline or *p*-anisidine + 2 N HCl; (C) Pd–C (10%); (D) 48% HBr; (E) Fremy's radical; (F) $NaBH_4$/ethanol.

benz[*a*]anthracene and benz[*a*]acridine, the carcinogenic properties of benz[*c*]acridine derivatives are found to be more,[3] mainly because of the electron-rich nitrogen atom's presence near the bay region of the polycycle. It is well established that the dihydrodiols of polyaromatic hydrocarbons are more carcinogenic than the parent hydrocarbons.[4]

We now wish to report a convenient method of synthesis for the title compounds which utilizes Fremy's salt for the oxidation of 3- or 8-hydroxybenz[*c*]acridine followed by borohydride reduction of the quinones.

Thus 6-methoxy-1-tetralone **1a** or 1-tetralone **1b** was subjected to the Vilsmeir–Haack reaction (phosphoryl chloride/dimethyl formamide) to produce the corresponding chloroaldehyde **2a** or **2b** in 75–80% yield (Scheme I). The resulting chloroaldehyde **2a** or **2b** was treated with aniline or *p*-methoxyaniline in ethanol to get the anil derivative **3a** or **3b** in 85–90% yield. The anil derivative **3a** or **3b** on brief heating at 250 °C readily cyclized to dihydrobenz[*c*]acridine **4a**[5] or **4b** as the only isolable product in 65–95% yield.

The enamino imine hydrochlorides cyclize during heating in only one way as evident from isolation of one compound, which is in agreement with related anils' thermal cyclizations.[1] The aromatization of dihydro compounds **4a,b** was performed by heating with Pd/C (10%) in *p*-cymene. 3-Hydroxybenz[*c*]acridine **6a** or 8-hydroxybenz[*c*]acridine **6b** was obtained from the corresponding methoxy derivative **5a** or **5b** by boiling with 48% HBr. Fremy's salt oxidation of phenols **6a,b** to quinones **7** or **8** was smooth and high yielding. Finally sodium borohydride reduction of the *o*-quinones produced the title compounds **9** or **10**.

The above synthesis provides a more convenient synthetic route to dihydrodiols **9** and **10** than previous methods.[6,7]

Experimental Section

General Notes. Fremy's salt (potassium nitrosodisulfonate) was prepared according to the method described by Zimmer.[8] Compounds **1a–4a** were prepared by following the method of J. K. Ray et al.[5] The NMR spectra were recorded on a Varian EM 390 spectrometer with tetramethylsilane as internal standard. All the melting points are uncorrected.

1-[(*p*-Methoxyphenyl)amino]-2-[((*p*-methoxyphenyl)imino)methyl]-3,4-dihydronaphthalene Hydrochloride (3b). The chloro formyl derivative **2b**[9] (8.0 g, 40 mmol) was added to a mixture of *p*-anisidine (10.4 g, 84 mmol) in ethanol (40 mL) and 3–4 N HCl (25 mL). The mixture was initially stirred for 2 h at room temperature, refluxed for 15 min, and cooled overnight in a refrigerator. The resulting solid was filtered, washed with alcohol, and dried to get 13.0 g (74.3%) of the hydrochloride **3b**. Recrystallization from ethanol and petroleum ether mixture afforded a yellow compound: mp 243–245 °C; IR (Nujol) ν_{max} 1615, 3350, 3440 cm^{-1}.

9-Methoxy-5,6-dihydrobenz[*c*]acridine (4b). The anil derivative **3b** (3 g, 7 mmol) was taken in a test tube and heated at 250 °C for 10 min. The resulting solid was washed several times with hot water, dried, and recrystallized (dichloromethane–petroleum ether) to get **4b** in excellent yield (1.8 g, 96.6%); mp 118 °C; ^1H NMR ($CDCl_3$) δ 2.9–3.15 (m, 4 H), 3.9 (s, 3 H), 7.0–8.5 (m, 8 H); MS, *m/e* 261 (M^+).

3-Methoxybenz[*c*]acridine (5a). 3-Methoxy-5,6-dihydronaphtho[1,2-*b*]quinoline **4a**[5] (375 mg, 1.43 mmol), 375 mg of Pd–C, and 7–8 mL of *p*-cymene were refluxed together for 2 h. The Pd–C was separated by filtration, and the filtrate, on removal of solvent under reduced pressure, afforded **5a** as a colorless solid: yield, 350 mg (94.1%); mp 123–124 °C (benzene–petroleum ether); ^1H NMR ($CDCl_3$) δ 3.95 (s, 1 H), 7.25–8.15 (m, 7 H), 8.40 (s, 1 H), 9.47 (d, 1 H). Anal. Calcd for $C_{18}H_{13}NO$: C, 83.4; H, 5.0; N, 5.4. Found: C, 83.1; H, 4.9; N, 5.2.

9-Methoxybenz[*c*]acridine (5b). The compound **4b** (405 mg, 1.5 mmol) on aromatization with Pd–C (200 mg) under the conditions as mentioned for **5a** afforded 315 mg (78%) of **5b**: mp 155 °C (benzene–petroleum ether); ^1H NMR ($CDCl_3$) δ 3.94 (s, 3 H), 7.14–8.46 (m, 10 H); MS, *m/e* 259 (M^+).

3-Hydroxybenz[*c*]acridine (6a). The methoxy derivative **5a** (200 mg, 0.772 mmol) was refluxed with 48% hydrobromic acid (15 mL). Initially the compound dissolved in HBr and about 45 min later a yellow solid separated out. Refluxing was continued

(2) Lacassague, A.; Buu-Hoi, N. P.; Daudel, R.; Zajdela, F. *Adv. Cancer Res.* **1956**, *4*, 315.
(3) Glatt, H. R.; Schwind, H.; Schechtman, L. M.; Beard, S.; Kouri, R. E.; Zajdela, F.; Croisy, A.; Perin, F.; Jacquignon, P. C.; Oesch, F. In *Short Term Test Systems for Detecting Carcinogens*; Norpoth, K. H., Garner, R. C., Eds.; Springer-Verlag: West Berlin, 1980.
(4) Harvey, R. G. *Acc. Chem. Res.* **1981**, *14*, 218.
(5) Ray, J. K.; Sharma, S.; Chatterjee, B. G. *Synth. Commun.* **1979**, *9*, 727.
(6) Schaefer-Ridder, M.; Englehardt, U. *J. Org. Chem.* **1981**, *46*, 2895.
(7) Lehr, R. E.; Kumar, S. *J. Org. Chem.* **1981**, *46*, 3675.
(8) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.
(9) Ziegenbein, W.; Lang, W. *Chem. Ber.* **1960**, *93*, 2433.

for 1.5 h more, and the mixture was cooled, diluted with water, and neutralized with $NaHCO_3$ solution. The solid separated was filtered, washed well with water, and air-dried. The crude product on recrystallization from benzene–methanol mixture afforded 170 mg (89.9%) of light yellow solid: mp 246–247 °C; ^1H NMR (DMSO-d_6) δ 7.28–7.48 (m, 2 H), 7.60–7.80 (m, 4 H), 8.28 (t, 2 H), 8.76 (s, 1 H), 9.28 (d, 1 H), 10.24 (s, 1 H). Anal. Calcd for $C_{17}H_{11}NO$: C, 83.2; H, 4.5; N, 5.7. Found: C, 83.1; H, 4.3; N, 5.5.

9-Hydroxybenz[*c*]acridine (6b). **5b** (191.1 mg, 7.3 mmol) on demethylation with 48% HBr as mentioned above for **6a** afforded 175 mg (96.8%) of **6b**: mp 208–211 °C; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.3–7.9 (m, 6 H), 8.1–8.2 (t, 2 H), 8.4 (s, 1 H), 9.3–9.4 (d, 1 H), 9.8 (s, 1 H); MS, *m/e* 245 (M^+).

3,4-Dioxo-3,4-dihydrobenz[*c*]acridine (7). To a stirred ice cooled solution of Fremy's salt (550 mg, 2.05 mmol) in 75 mL of water was added 0.16 M KH_2PO_4 buffer (6–8 mL). To this was added a solution of the phenol **6a** (100 mg, 0.408 mmol) in 25 mL of methanol–THF mixture and stirring continued for 2 h at 0–15 °C. The mixture was left in refrigerator for overnight. The green solid separated was filtered, washed well with water, and air-dried: yield, 90 mg (85%); mp of the crude product 266–268 °C dec; IR (Nujol) ν_{max} 1608, 1637, 1658 cm^{-1}.

8,9-Dioxo-8,9-dihydrobenz[*c*]acridine (8). The phenol **6b** (110.6 mg, 0.45 mmol) on oxidation with Fremy's salt (470 mg, 1.75 mmol) in a methanol (20 mL) and water (15 mL) mixture, after usual workup, afforded 88.4 mg (75.9%) of the quinone **8** as a reddish brown solid: mp >300 °C; IR (Nujol) ν_{max} 1675, 1700 cm^{-1}; MS, *m/e* 259 (M^+).

3,4-Dihydro-3,4-dihydroxybenz[*c*]acridine (9). To a stirred suspension of the quinone **7** (30 mg, 0.116 mmol) in 15 mL of ethanol was added 75 mg of $NaBH_4$ in four batches. Stirring was continued for 48 h at room temperature in presence of air, ethanol was evaporated at room temperature, and the residue was diluted with water and extracted with ethyl acetae. The usual workup and removal of solvent gave 28 mg of crude solid. This on purification by preparative TLC (silica gel) using ethyl acetate–petroleum ether mixture as eluent afforded 6 mg (19.7%) of the dihydrodiol **9** as a yellow solid (mp and mixed mp with authentic sample[6] was 200–201 °C) and 22 mg of phenol **6a** (mp and mixed mp with previous sample 246–247 °C). The dihydrodiol **9** was also characterized by ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 4.47 (m, 1 H, H-3), 4.85 (m, 1 H, H-4 6.22 (m, 1 H, H-2), 7.5–8.3 (m, 7 H, H-1 and H-5, 6, 8–11), 9.09 (s, 1 H, H-7) ($J_{1,2}$ = 10 Hz) [lit.[6] ^1H NMR (DMSO-d_6, D_2O) δ 9.13 (s, 1 H, H-7), 7.9 (d, 1 H, H-1), 7.4–8.3 (m, 6 H, H-5, 6, 8–11), 6.28 (dd, 1 H, H-2), 4.89 (m, 1 H, H-4), 4.50 (m, 1 H, H-3) ($J_{1,2}$ = 9.9 Hz, $J_{2,3}$ = 2.7 Hz, $J_{3,4}$ = 10.2 Hz)].

8,9-Dihydroxy-8,9-dihydrobenz[*c*]acridine (10). The quinone **8** (100 mg, 0.4 mmol) in ethanol (6 mL) on reduction with $NaBH_4$ (82 mg) as per conditions mentioned above afforded 80 mg (80%) of the dihydrodiol **10**. It was purified by preparative TLC (silica gel; ethyl acetate–petroleum ether): mp 190–191 °C (lit.[7] mp 177–179 °C). The dihydrodiol **10** was characterized by ^1H NMR and its diacetate (pyridine/Ac_2O) showed a spot identical with that of an authentic sample[7] in TLC: ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 4.6 (d, 1 H), 5.0 (d, 1 H), 6.4 (d, 1 H), 6.8 (d, 1 H), 7.6–8.0 (m, 5 H), 8.3 (s, 1 H), 9.2–9.3 (d, 1 H) [lit.[7] ^1H NMR δ 4.46 (H-9), 4.88 (H-8), 6.43 (H-10), 6.80 (H-11), 7.66–8.14 (5 H), 8.38 (H-7), 9.06–9.28 (H-1).

Acknowledgment. We express our deep sense of gratitude to Dr. M. Schafer-Ridder, West Germany, for providing us with 1 mg of a sample of the dihydrodiol **9** for comparison and also to Dr. R. E. Lehr, U.S.A., for sending us few milligrams of the diacetae of the compound **10**. Thanks are also due to Dr. P. N. Moza, West Germany, for a few mass spectra and Dr. Malay Ghosh, U.S.A., for the NMR of **10**. C.S.I.R., New Delhi, deserves special thanks for financial assistance.

Registry No. **1a**, 1078-19-9; **1b**, 529-34-0; **2a**, 72019-91-1; **2b**, 3262-03-1; **3a**, 111378-92-8; **3b**, 111378-93-9; **4a**, 72019-93-3; **4b**, 111351-44-1; **5a**, 111351-45-2; **5b**, 111351-46-3; **6a**, 111351-47-4; **6b**, 111351-48-5; **7**, 111351-49-6; **8**, 111378-94-0; **9**, 77305-76-1; **10**, 85617-30-7; $PhNH_2$, 62-53-3; *p*-anisidine, 104-94-9.

^{13}C NMR Investigations on the Structure of α-Keto Acids in Aqueous Solution

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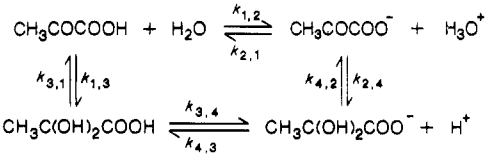
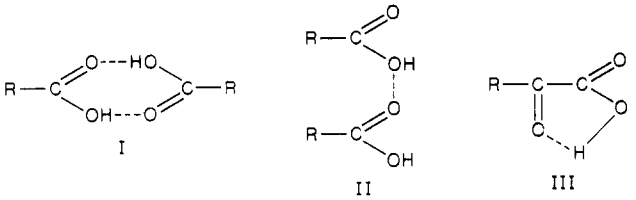
α-Keto acids play a central role as intermediates in the metabolism of carbohydrates and proteins, and their structure and reactivity under physiological conditions are therefore of interest. Pyruvic acid exists in aqueous solution as a mixture of four species: the hydrated and nonhydrated undissociated acids and anions.[1-4] In the equilibria among these species, $k_{1,2}$, $k_{2,1}$, $k_{3,4}$, and $k_{4,3}$ are fast on the NMR time scale relative to the ^{13}C chemical shifts.



The thermodynamics and kinetics of the hydration of pyruvic acid have been widely investigated, and the hydration equilibrium has been determined by UV absorption measurements at 320 nm and by ^1H NMR spectroscopy.[1,2,4,5]

Unsubstituted organic acids dimerize in nonpolar (I) and polar (II) solvents, whereas α-keto acids exist largely as monomers owing to formation of an intramolecular hydrogen bond (III) with the α-carbonyl group.[6-8] In aqueous solution, α-keto acids exist as monomers.



We here report on the ^{13}C chemical shifts of pyruvic acid and 2,2-dihydroxypropanoic acid in the pD range 2.0–5.5. The change in the hydration equilibrium was determined from the areas of the methyl C peaks of both molecules. Furthermore, the ^{13}C chemical shifts indicate an unusually high sensitivity of the α-carbon atom of pyruvic acid toward the prototropic equilibrium of the neighboring carboxyl group.

The ^{13}C NMR spectrum of a solution of pyruvic acid in D_2O at pD 2.0 contains six peaks which can be assigned to the carbon atoms of pyruvic acid and 2,2-dihydroxypropanoic acid. The ^{13}C chemical shifts in the pD range 2.0–5.5 ppm indicate a shift of the hydration equilibrium toward the keto form with increasing pD. This shift was determined from the ratio of the methyl carbon peak areas, which was 1.18 at pD 2.0 and 0.17 at pD 4.0 for 0.5 M pyruvic acid. The literature reports peak area ratios of 1.77 for 0.4 M pyruvic acid[9] and 1.67 for 2.0 M pyruvic acid,[2] in both cases for the undissociated acid.

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