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FLUORESCENT AND PHOSPHORESCENT PYRIMIDINE LABELS

α-DIKETONE DERIVATIVES OF URACIL AND THYMINE

YONG J. LEE, WILLIAM A. SUMMERS and JOHN G. BURR*
Department of Chemistry, University of Oklahoma, Norman, OK 73019, U.S.A.

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Abstract—The syntheses of 1-(3,4-dioxopentyl)uracil (V), 1-(2,3-dioxobutyl)uracil (XIIa), 1-(2,3-dioxobutyl)-3-methyluracil (XIIb) and 1-(2,3-dioxobutyl)thymine (XIIc) are described. These are the first compounds to be prepared which have α-diketone functions attached to biologically important pyrimidines. Preparation of the dioxopentyluracil was by oximation of 1-(4-oxopentyl)uracil, and of the dioxobutyl compounds was by alkylation of the appropriate pyrimidine with the dimethoxy ketal of bromobiacetyl, followed by hydrolysis under special conditions. The characteristics of the absorption and emission spectra in various solvents are presented and discussed. Dioxopentyl uracil exhibits both phosphorescence and fluorescence at room temperature; the dioxobutyl pyrimidines are fluorescent but non-phosphorescent under the same conditions. The fluorescence quantum yields of all four compounds are about 0.2%, similar to those of biacetyl or 2,3-pentanedione.

Earlier reports[1,2] from this laboratory have discussed the preparation of bichromophoric compounds containing pyrimidines and fluorescent dyes and the mode of intramolecular energy transfer between pyrimidines and dye chromophores.

These bichromophoric compounds proved to be an excellent system for studying the pyrimidine excited state,[3] particularly the excited singlet state. However, the lack of phosphorescence of the available fluorescent dyes at room temperature was a major drawback in an effort to understand both the excited singlet and triplet state of the pyrimidines.

In a continuation of this effort, we now report the preparation of a new series of bichromophoric compounds containing uracil and thymine with α-diketone side chains; here the chromophores are linked in a non-conjugative manner.

Short chain α-diketones are unique in that they exhibit phosphorescence in solution at room temperature. The spectral distribution and the molar extinction coefficients of the diketone make possible selective excitation of either chromophore. The diketones should be a good excitation energy trap because of the low energy level of the excited state.

EXPERIMENTAL

Materials. Uracil, thymine, 3-methyluracil were obtained either from Sigma Chemicals or Cyclochemicals. Bromobiacetyl[4] and its methylketal, 5-bromo-2-pentanone[5] and its ethylene ketal[6] were prepared according to literature procedures. Mallinckrodt silica gel CC-4 was used for column chromatography and thick layer chromatographic plates were prepared with Brinkman PF-254 silica gel. Eastman silica gel on polypropylene sheets were used for analytical TLC works.

Spectra. Absorption spectra were recorded either on Perkin-Elmer Hitachi 124 or Cary 118 spectrophotometer, and fluorescence and phosphorescence spectra were taken on Perkin-Elmer MPF-3 spectrofluorometer equipped with ratio mode recording. Corrections of fluorescence spectra were made according to previous descriptions.[3] Fluroescence spectra shown in Figs. 3 and 4 are not corrected.

Synthesis

1-(4-*Oxopentyl*)*uracil* (III). Alkylation of uracil with 5-bromo-2-pentanone in DMSO/K_2CO_3[7,8] was unsuccessful, probably because of dehalogenation of the ketone. However, the ethylene ketal of the ketone[6] 2-methyl-2-(3-bromopropyl)1,3-dioxolane, gave a good yield of the corresponding alkylated uracil. Since the uracilyl ketal was slow to crystallize, it was hydrolyzed directly to the uracilyl ketone which did crystallize.

Uracil (5.0 g, 45 mmole) and 2-methyl-2-(3-bromopropyl)-1,3-dioxolane (2.07 g, 10 mmole) were dissolved in 100 ml dry DMSO and treated with anhyd K_2CO_3 (6.35 g, 46 mmole) for 72 hr. After filtering the gelatinous ppt, the filtrate was evaporated to a light yellow viscous oil (1.5 g). NMR ($CDCl_3$/TMS) δ 1.33 (S, 3H), 1.75 (m, 4H), 3.46 (m, 2H), 3.96 (S, 4H), 5.73 (d, 1H, J = 8 Hz uracil (5)-H), 7.25 (d, 1H, J = 8 Hz, uracil-(6)-H).

This material after silica gel chromatography would not afford a solid and was then hydrolyzed with 10% aqueous formic acid on a steam bath for 30 min. The mixture was concentrated to a gum, rendered anhydrous by repeated evaporation of pyridine followed by evaporation of abs. EtOH. The remaining oil was chromatographed in silica gel in $CHCl_3$ affording 1.25 g white crystalline solid, 65%. m.p. 97.5–99°, IR 3.3, 5.88, 5.95, 6.77, 7.15, 7.55, 7.93, 8.45, 9.45, 11.1 and 12.2 μ; UV (MeOH) 266 nm (+9.980); NMR ($CDCl_3$/TMS) δ 1.97 (m, 2H, 2'-CH_2), 2.17 (S, 3H, 5'-CH_3), 2.54 (t, 2H, J = 6 Hz, 3'-CH_2), 3.77 (t, 2H, J = 6 Hz, 1'-CH_2), 5.70 (d, 1H, J = 7.8 Hz, U(5)-H and 6.33 (S, 1H, N(3)-H) and 7.24 (d, 1H, J = 7.8 Hz, U(6)-H); m/s (70 eV) *m/e* (rel. ab.) 196(14), 153(10), 139(100), 126(14), 113(14), 113(14), 96(76) and 85(28). (Found: C, 55.15; H, 6.26. Calc. for $C_9H_{12}N_2O_2$; C, 55.10; H, 6.12).

1-(3,4-*Dioxopentyl*)*uracil* (V) *and* 1-(3-*oximino*-4-*oxopentyl*)*uracil* (IV). The nitrosation of ketones[9–11] is the oldest reported method for preparation of α-diketones. This was the method which enabled us to prepare the dioxopentyluracil, but several features of the method seem unique and unexpected. Treatment of the 4-oxopentyluracil with an alkyl nitrite, did not give the diketone in one step, even with an excess of the nitrite for 96 hr at room temp. Only the oximino ketone resulted (plus a minor product which was not the diketone).

Diketone resulted only when the oximinoketone was isolated and treated again with the alkyl nitrite. Hydrolysis of the oximinoketone to the diketone could not be accomplished, and transoximation was also unsuccessful. This two step conversion of the ketone to the diketone has precedent. In one of the early papers, Manasse[9] reported the preparation of diketone by treatment of the oximinoketone with alkyl nitrite and a trace of acid, to give N_2O and the diketone.

1-(4-*Oxo*-3-*oximinopentyl*)*uracil* (IV). 1-(4-Oxopentyl)uracil (1.0 g, 5.1 mmole) was treated in 100 ml isopropanol with 0.5 ml conc. HCl and propyl nitrite (1.0 g, 11.8 mmole) for 18 hr at room temp. The resulting crystalline solid was filtered off, washed with

isopropanol and air dried affording 962 mg of white crystalline solid, 80%; m.p. 201.5–203°; IR, 3.06, 3.18, 3.3, 3.5, 6.0 (broad), 6.78, 7.3, 7.85, 8.33, 9.18, 9.72, 10.1, 10.2, 12.05, 13.0 and 16.4 μ; UV (MeOH) 264 nm (ε 10,500); NMR (pyridine-d_5/TMS) δ 2.42 (S, 3H, 5'-H), 3.22 (t, 2H, J = 7 Hz, 3'-H), 4.12 (5, 2H, J = 7 Hz, 1'-H), 5.75 (d, 1H, J = 8 Hz, U(5)-H), 7.45 (d, 1H, J = 8 Hz, U(6)-H); m/s (at 70 eV) *m/e* 224(20), 181(11), 165(11), 140(17), 126(47) and 113(100). (Found: C, 48.06; H, 4.87; N, 18.54. Calc. for $C_9H_{11}N_3O_4$: C, 47.99; H, 4.92; N, 18.66%).

1-(3,4-*Dioxopentyl*)*uracil* (V). 1-(4-Oxo-3-oximinopentyl)uracil (354.5 mg, 1.57 mmole) was treated with amyl nitrite (5 g) at 100° for 5 hr. The solvent and volatile by-products were evaporated at reduced pressure. The resulting viscous yellow oil was chromatographed on silica gel with $CHCl_3$–1% MeOH, affording 88 mg of a yellow crystalline solid (from ether), 27%; m.p. 115–116°; IR 2.93, 3.15, 3.24, 3.29, 3.58, 5.15 (weak), 6.17 (intense, broad), 6.9, 7.2, 7.45, 8.15, 8.45, 8.55, 9.5, 11.2, 11.8, 12.2 and 16.4 μ. UV (MeOH) 263 nm (ε 10,000) fluorescence max, 460 nm; NMR (DMSO-d_6/TMS) δ 2.23 (S, 3H, 5'-H), 3.04 (t, 2H, J = 6.2 Hz, 2'-H), 3.9 (t, 2H, J = 6.2 Hz, 1'-H), 5.53 (d, 1H, J = 8 Hz, u(5)-H and 7.65 (d, 1H, J = 8 Hz, U(6)-H); m/s 210(2), 182(7), 167(64), 140(50), 125(54), 112(100), 98(53). (Found: C, 51.29; H, 4.70. Calc. for $C_9H_{10}N_2O_4$: C, 51.43; H, 4.80%).

Attempted preparation of 1-(3,4 - *dioxopentyl*)*uracil from* 1-(4-*oxo*-3-*bromopentyl*)*uracil* (VI). Oxidative hydrolysis of VI was envisaged as one possible route to the uracilyl diketone. However, preparation of the bromoketone from 1-(4-oxopentyl)uracil proved unexpectedly difficult. Standard methods for preparation of bromoketones are not usable because of interference by the uracil moiety. However, cupric bromide in EtOAc[12,13] oxidized it to the desired bromoketone, with no bromine incorporation into the uracil ring. The yield was unfortunately so low that further elaboration of this synthetic pathway was precluded.

1-(4-*Oxo*-3-*bromopentyl*)*uracil* (VI). 1-(4-Oxopentyl)uracil (500 mg, 2.55 mmole) was dissolved in EtOAc and treated with cupric bromide (650 mg, 5.5 mmole) for 24 hr at room temp. The reaction was filtered and concentrated to a small volume at reduced pressure. The reaction was then applied to 4 preparative tlc plates and developed 4 times in EtOAc. The product was eluted with abs. EtOH, which afforded a viscous oil after removing solvents. The oil was dissolved in 500 ml ether which afforded 120 mg white crystalline solid by slow evaporation, 17%, m.p. 120–122°; IR 3.16, 3.28, 5.90, 6.02, 6.15, 6.92, 7.09, 7.35, 7.42, 7.70, 8.13, 12.5 and 16.7 μ; UV (MeOH) 267 nm (ε 10,200); NMR ($CDCl_3$/TMS) δ 2.37 (S, 1H, 5'-H), 2.33 (m, 1H, 3'-H), 3.93 (m, 2H, 2'-H), 4.36 (5, 2H, J = 7.4 Hz, 1'-H), 5.7 (d, 1H, J = 8 Hz, uracil-(5)-H), 7.2 (d, 1H, J = 8 Hz uracil-(6)-H); m/s (at 70) *m/e* 276(0.01), 232–234(5), 195(15), 153(26), 139(47), 126(100), 113(75), 96(85). (Found: C, 38.83, H, 3.92. Calc. for $C_9H_9BrN_2O_3$: C, 39.28; H, 4.03%).

Attempted preparation of 1-(2,3-*dioxobutyl*)*uracil* (XIIa) *from* 2-*bromoacetyl*-2-*methyl*-1,3-*dioxalane* (VIII). Direct alkylation of uracil with bromobiacetyl in a variety of systems[7,8] was unsuccessful, owing to the lability of the C–Br bond. Alkylation of uracil with the bisdioxalane of biacetyl, 2,3-di(bismethylenedioxy)-1-bromobutane, was also unsuccessful, owing to the unfavorable steric conformation of the bisdioxalane. Alkylation of uracil with the monodioxalane of bromobiacetyl, 2-bromoacetyl-2-methyl-1,3-dioxalane, was successful in DMSO/K_2CO_3 (Brown's system)[9] but it was found impossible to hydrolyze the ketal to diketone in any of the several acidic systems investigated (refluxing acetone/TsOH, benzophenone/TsOH and 120°, 60% aqueous formic acid at 100°, constant boiling HCl).

2,3-*Di*(*bismethylenedioxy*)-1-*bromobutane* (VII) *and* 2-*bromoacetyl*-2-*methyl*-1,3-*dioxalane* (VIII). Bromobiacetyl (20 g, 121 mmole) was dissolved in 100 ml dry benzene and 30 ml ethylene glycol added. This mixture was refluxed with 10 mg toluenesulfonic acid until the yellow color dissipated. The reaction was then partitioned with 150 ml sat $NaHCO_3$ aq and washed with water. The benzene was evaporated to a slurry containing a crystalline solid and a high boiling liquid. The solid VII was recovered by trituration with hexane, 17.4 g crude m.p. 110–118°. This could be recrystallized from EtOAc/hexane; m.p. 112.5–113.5; IR 3.14, 3.35, 3.38, 3.45, 3.46, 6.77, 6.95, 7.25, 7.72, 7.87, 7.91, 8.13, 8.44, 8.73, 9.09, 9.82, 10.1, 11.55, 11.98, 14.6 and 15.6 μ; NMR (acetone-d_6/TMS) δ 1.37 (S, 3H), 2.97 (S, 2H) and 3.76 (m, 8H). (Found: C, 37.86, H, 5.04; Br, 31.59. Calc. for $C_8H_{13}BrO_4$: C, 37.96; H, 5.18; Br, 31.57%).

The oil (VIII) was recovered from the hexane wash by removal of the hexane at reduced pressure. This was, after filtration of some residual diketal, pure for analysis, b.p. (0.015 torr) 49.5°; IR 3.34, 3.44, 5.71, 6.75, 6.89, 7.25, 8.20, 8.93, 9.75, 10.75, 11.2, 11.58 and 15.3 μ; NMR ($CHCl_3$-d/TMS) δ 1.58 (S, 3H), 4.24 (S, 4H), 4.45 (S, 2H). (Found: C, 34.48; H, 4.09; Br, 37.82. Calc. for $C_6H_9O_3Br$: C, 34.45; H, 4.30; Br, 38.27%).

1-[2-(*Dimethylenedioxy*)-2-*oxobutyl*]*uracil* (IX). Uracil (5.0 g, 45 mmole) was alkylated in the manner described above with 2-methyl-2-(bromoacetyl)-1,3-dioxolane and after purification by preparative tlc afforded 966 mg crystalline solid, 40% yield, m.p. 118.5–119°; IR 3.32, 3.55, 5.67, 5.88, 6.0, 6.79, 7.1, 7.19, 7.4, 8.0, 8.29, 9.67, 11.2 and 13.0 μ; UV (MeOH) 265 (ε 10,400); NMR (pyr-d_5/TMS) δ 1.57 (S, 3H, 4'-H), 3.93 (S, 4H, ketal), 5.10 (S, 2H, 1'-H), 5.25 (d, 1H, J = 7.4 Hz, U(5)-H), 7.15 (S, 1H, N(3)-H), 7.5 (d, 1H, J = 7.4 Hz, U(6)-H); m/s (at 70 eV) *m/e* 241 (0.3), 181(9.4), 136(9.7), 125(1.6), 118(9.7), 99(6) and 87(100). (Found: C, 49.92; H, 5.15. Calc. for $C_{10}H_{12}N_2O_5$: C, 49.99; H, 5.04%).

3,3-*Dimethoxy*-1-*bromo*-2-*butanone* (X). Bromobiacetyl (8 g, 0.05 m), dissolved in 20 ml MeOH (dried by distillation from magnesium methoxide) containing a suspension of 0.1 g NH_4Cl, was refluxed for 1 hr, let stand overnight at room temp., neutralized with sat $NaHCO_3$ aq, and the MeOH removed at reduced pressure. The residue was extracted with benzene. The dried benzene soln was evaporated at reduced pressure, and the residue distilled under vacuum, b.p. 40°, (1 mmHg) yield 3.2 g (31%). NMR ($CDCl_3$/TMS), δ 1.42 (S, 3H), 3.21 (S, 6H), 4.33 (S, 2H); IR 3.36, 3.42, 5.73, 6.85, 7.25, 6.36, 8.57, 7.79, 9.50, 9.78, 11.0, 15.25 μ; UV_{max} (acetonitrile 270 nm (ε 32). (Found: C, 34.27; H, 5.27. Calc. for $C_6H_{11}BrO_3$: C, 34.12; H, 5.21%).

1-(3,3-*Dimethoxy*-2-*oxobutyl*)*uracil* (XIa). A mixture of 5 g uracil, 2.8 g (0.013 m) of X and 5.5 g K_2CO_3 in 100 ml of DMSO immediately colored to a dark orange, it was stirred for 48 hr, until tlc analysis showed the reaction to be complete. The dark brown gelatinous soln was filtered, and the filtrate reduced under vacuum to a syrup; the solid on the filter was suspended in water, and the mixture extracted with EtOAc. The syrupy filtrate was suspended in 50 ml water and the mixture extracted with EtOAc, 3 × 100 ml. The combined EtOAc extracts were dried and concentrated at reduced pressure. The semi-solid residue was recrystallized from water to give 1.7 g (45%) of m.p. 170°. NMR (pyridine-d_6/TMS) δ 1.52 (S, 3H), 3.30 (S, 6H, $-OCH_3$), 5.23 (S, 2H), 5.95 (d, 1H, 5-H), 7.66 (d, 1H, 6-H); IR 3.18, 3.29, 3.39, 3.53, 3.73, 5.97, 6.84, 7.00, 7.20, 7.41, 7.97, 8.25, 8.60, 8.80, 9.05, 9.51, 9.70, 10.5, 11.1, 12.1 μ. (Found: C, 49.59, H, 5.77. λ_{max} 260 (ε 10,200). Calc. for $C_{10}H_{14}N_2O_5$: C, 49.59; H, 5.79%).

1-(2,3-*Dioxobutyl*)*uracil* (XIIa). A soln of XIa (280 mg) in 3 ml 50% formic acid (1.1 mM) was refluxed for 10 min; the soln was then frozen in dry ice-acetone, and freeze-dried to 250 mg of a white powder which was stable at room temp. This white powder had an NMR spectrum corresponding to the hemi-hydrate, Ura–CH_2–$C(OH)_2$–CO–CH_3. The flask containing the white powder was heated under vacuum with a heat gun until the powder became a homogeneous yellow color, the amount was reduced to 227 mg which corresponds to loss of 18 mg water (1 mM). The yellow diketone was recrystallized from EtOAc to give 172 mg (85%), m.p. 159°. NMR (DMSO-d_6/TMS) δ 2.35 (S, 3H), 5.0 (S, 2H), 5.60 (S, 1H, (6)-H), 7.50 (S, 1H, (5)-H). IR 3.18, 3.22, 3.31, 3.39, 3.47, 3.54, 3.63, 5.73, 5.96, 6.80, 6.96, 7.20, 7.40, 7.91, 8.18, 8.22, 9.05, 9.30, 11.0, 12.0, 12.2, 12.75, 13.3 μ. UV λ_{max} 260 (ε 10,200), 410 (ε 31.5) nm. (Found: C, 48.94; H, 4.19. Calc. for $C_8H_8N_2O_4$: C, 48.98; H, 4.08%).

1-(3,3-*Dimethoxy*-2-*oxobutyl*)3-*methyluracil* (XIIb). 3-methyluracil (0.5 g, 0.004 m), 0.5 g anhyd K_2CO_3, and 1.2 g (0.006 m) of X in 15 ml dimethylsulfoxide was stirred for one day at ambient temp. Through the similar work-up procedure as is described in the preparation of XIa, an oil product was obtained, and this oil slowly solidified. The solid was purified by chromatographing on thick layer plate of silica gel in an EtOAc solvent.

The slower band on thick layer was unreacted 3-methyluracil and the product was in a faster moving band. After taking off the fast moving band, the product was extracted in EtOAc and removing solvent yielded an oil which solidified very slowly (24 hr) by setting at room temp. (0.5 g, 50% yield), m.p. 90–92°. UV (acetonitrile) 260 nm $\epsilon_{260} = 9950$; NMR ($CDCl_3$/TMS) δ 1.42 (S, 3H), δ 3.50 (S, 6H), δ 3.35 (S, 3H), δ 4.80 (S, 2H), δ 5.80 (d, 1H, J = 8 Hz), δ 7.0 (d, 1H, J = 8 Hz). (Found: C, 51.46; H, 6.17. Calc. for $C_{11}H_{16}N_2O_5$: C, 51.56; H. 6.25%).

1-(2,3-*Dioxobutyl*)-3-*methyluracil* (XIIb). 113 mg of XIb in 1 ml trifluoroacetic acid was warmed at 70° for 15 min and the solvent was evaporated under vacuum. The residue yellow oil was crystallized from 1:1 mixture of EtOAc and cyclohexane yielding 78 mg yellowish crystals (84% yield), m.p. 95–96°. UV (acetonitrile) 260 nm, 415 nm, $\epsilon_{260} = 9510$, $\epsilon_{415} = 36$; NMR ($CDCl_3$/TMS) δ 2.40 (S, 3H), δ 3.30 (S, 3H), δ 4.90 (S, 2H), δ 5.80 (d, 1H, J = 8 Hz), δ 7.0 (d, 1H, J = 8 Hz). (Found: C, 51.41, H, 4.75. Calc. for $C_9H_{10}N_2O_4$: C, 51.43; H, 4.76%).

1-(3,3-*Dimethoxy-2-oxobutyl*)*thymine* (XIc). 5 g of thymine (0; 04 m) 3 g of anhyd K_2CO_3, 3 g of X (0.014 m) was mixed in 100 ml dry DMSO and stirred for 2 days at room temp. The mixture was worked up in a similar way as in the case of IXa yielding 1.5 g crude solid, m.p. = 180° (42% yield). Tlc showed about 10% thymine was in the crude product and chromatographing on thick layer plate of silica gel with EtOAc removed all the thymine, and the collected product was recrystallized from small volume of EtOAc to give yellow crystals showing a single spot on tlc (SiO_2, ethylacetate). M.p. 184–186°. UV (acetonitrile) 265 nm (ϵ_{265} = 9500); NMR (DMSO-d_6/TMS), δ 1.35 (S, 3H), δ 1.75 (S, 3H), δ 3.20 (S, 6H), δ 4.80 (S, 2H), δ 7.40 (S, 3H broad). (Found: C, 51.42; H, 6.32; N, 10.85. Calc. for $C_{11}H_{16}N_2O_5$: C, 51.56; H, 6.25; N, 10.94%).

1-(2,3-*Dioxobutyl*)*thymine* (XIIc). 110 mg of XIc was dissolved in 2 ml of 50% formic acid and heated up to 80–100° in oil bath for 10 min and the solvent was evaporated under vacuum maintaining the warm oil bath. Crude yellow residue solid was recovered, (80 mg, 90% yield) m.p. = 180–182°, and the solid was recrystallized from EtOAc, m.p. = 184–185°. UV (acetonitrile) 265, 415 nm. $\epsilon_{265} = 10{,}700$, $\epsilon_{415} = 38$; NMR (DMSO-d_6/TMS), δ 1.78 (S, 3H), δ 2.30 (S, 3H), δ 4.90 (S, 2H), δ 7.30 (S, 1H). (Found: C, 51.39; H, 4.87; N, 13.19. Calc. for $C_9H_{10}N_2O_4$: C, 51.43; H, 4.76; N, 13.33%).

Attempted preparation of 1-(3,4-*dioxopentyl*)*uracil from the hydrazide of* 3-(1-*thyminyl*)*propionic acid.* An old procedure[14] for preparation of diketones is the oxidation with Hg^{2+}/I_2 of an unsymmetrical hydrazide to a diacylazine, followed by CuO catalyzed thermolysis of the azine to a diketone. Application of this procedure to the hydrazide of 3-(1-thyminyl)propionic acid was unsuccessful.

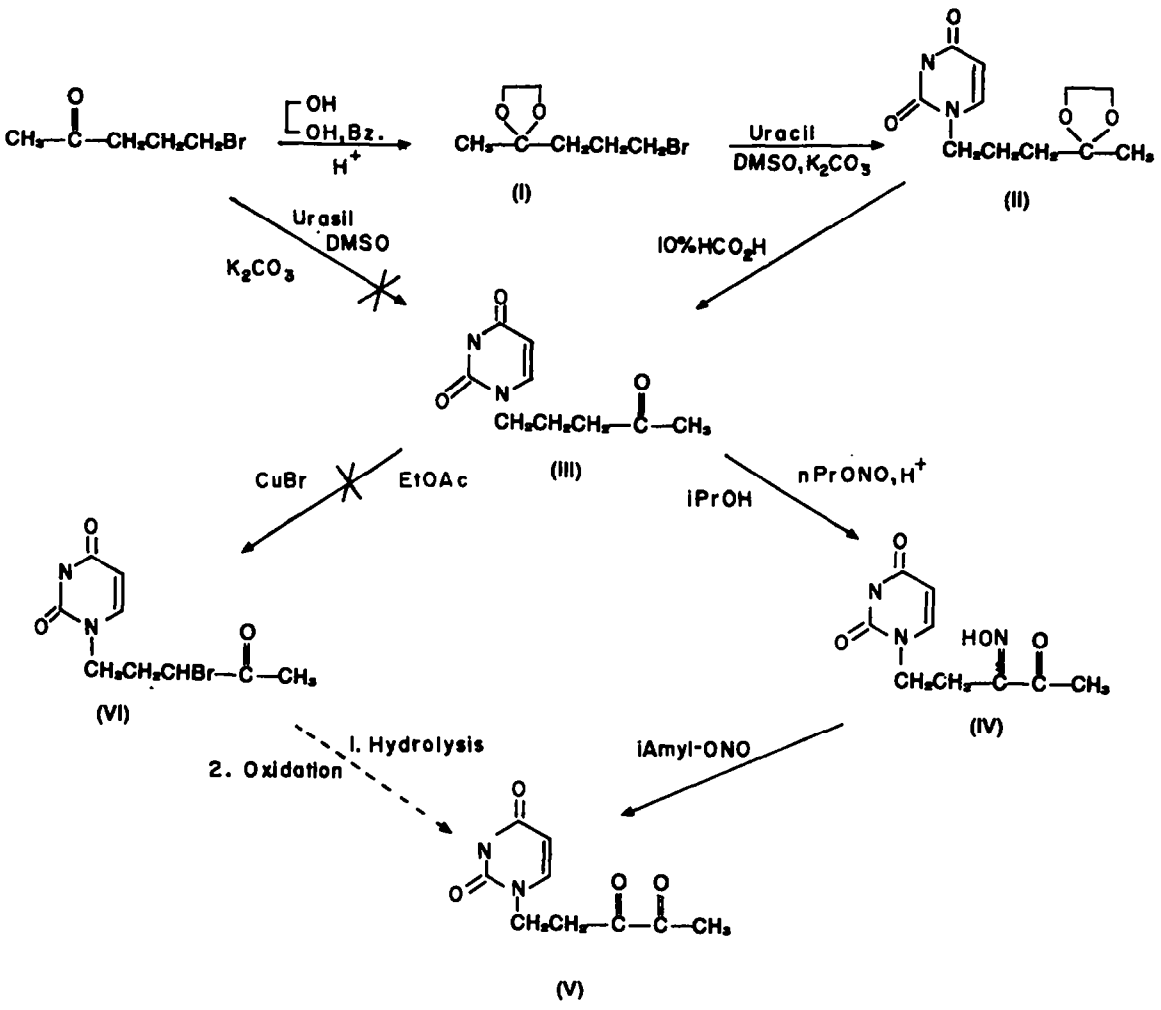
1-[3-*Thyminyl*)*propionyl*]-2-*acetylhydrazide.* 3-(1-Thyminyl)propionic acid (416 mg, 2.1 mmole) was suspended in dry acetonitrile and then treated with oxalyl chloride (180 μl, 4 mmole) for 1 hr. All the solid dissolved and the mixture was rapidly added to a soln of acetylhydrazine in acetonitrile (165 mg/10 ml) and allowed to stand overnight. The resulting white crystalline solid was filtered and air dried, 363 mg, m.p. > 325°. The product was soluble only in hot water. IR 3.1, 3.26, 3.52, 4.74, 5.82, 5.95, 6.17, 6.25, 6.66, 7.35 and 8.2 μ. UV_{max} (water) 265 nm (ϵ 9,800); NMR (DMSO-d_6-pyr-d_5 1:1/v/v with TMS) δ 1.98 (S, 3H, 5-CH_3), 2.07 (S, 3H, acetyl-CH_3), 2.84 (T, 2H, J = 6.8 Hz, 2'-H), 4.12 (t, 2H, J = 6.8 Hz, 1'-H), 7.44 (S, 1H, 6-H). (Found: C, 47.07, H, 5.45. Calc. for $C_{10}H_{14}N_4O_4$: C, 47.24; H, 5.51%).

RESULTS AND DISCUSSION

Syntheses. The goal of the syntheses described here was to attach 2,3-butanedione and 3,4-pentanedione sidechains to the 1-position of uracil and thymine. The syntheses proved to be difficult and so they are given in considerable detail in the Experimental; unsuccessful synthetic routes are simply listed without detail. Outlines of the synthetic paths followed, including unsuccessful ones as well as the successful ones, are shown in Schemes 1 and 2 where dashed lines indicate speculative pathways. Some interesting structural effects were observed during the course of this synthetic work, and are discussed here. Preparation of the pyrimidyl dioxobutyl derivatives was attempted initially with the bis-dioxolane derivative of bromobiacetyl, but it was found that this halide was so sterically hindered that alkylation of either uracil or thymine was impossible. This alkylation proceeded well with the mono-dioxolane derivative (IX), and preparation of the mono-dioxolane derivative of 1-(2,3-dioxobutyl)uracil was successful. However, acid hydrolysis of this compound proved very difficult, and the hydrolysis under the very stringent conditions necessary gave a product which did not appear to be the desired diketone. This difficulty is probably caused by the stability of the cyclic ketal relative to the open chain ketal.

Guided by this observation, we found that bromobiacetyl could be easily ketalized with methanol, giving a good yield of the mono-ketal (X). Ketalization of both carbonyl groups occurred only to a very small degree; furthermore, it is interesting to note that while hemiketalization of bromobiacetyl in methanol solution[15] occurs at the 2-position, full ketalization takes place only at the 3-position ($BrCH_2COC(OCH_3)_2CH_3$). This was proved by the NMR spectra; it probably provides evidence for the importance of a carbonium ion intermediate $[(BrCH_2{-}CO{-}\overset{+}{C}(OCH_3){-}CH_3]$[17] in the rate-determining step of ketalization, whereas hemiketal formation is governed mainly by the inductive effect of the neighboring bromine. In contrast to the mono-dioxolane ketal, the dimethoxy ketal was rapidly hydrolyzed; warm 50% formic acid quantitatively converted it to the diketone, both in the case of the pyrimidinyl butanediones (XIb, c) and also in the case of the corresponding open chain diketones. Furthermore, uracil, 3-methyluracil and thymine were smoothly alkylated by these dimethoxyketals in all cases (Experimental).

Progress in the hydrolysis of the dimethoxy ketals, XIa–c, was readily shown by the appearance of a visible absorption band (*ca.* 410 nm) or by the presence of a yellow color where these colorless compounds are dissolved in acidic media. All acids tried (surfuric, hydrochloric, formic acid) were effective even at dilute concentrations, however, the rate of hydrolysis is slower in dilute acid. The reaction can best be monitored by observing the NMR spectral change upon hydrolysis. Mild heating (80°) greatly facilitates the reaction and thus a 100 mg scale reaction can be completed in a few minutes. However, approximately 70% of the diketone exists as a hemihydrate in aqueous media and ketone/hydrate equilibria is sensitive to temperature with hydrate formation favored at lower temperature.[15,16] In fact dioxobutyluracil hemihydrate (XIIIa) was obtained by lyophilization of the reaction mixture. The mixture in formic acid was a deep yellow; lyophilization yielded a colorless solid residue which did not possess the typical visible absorption of the diketones. By heating under vacuum, the colorless solid (XIIIa) was converted to a yellow solid. Loss of mass during this conversion corresponded to one mole water per each mole of the compound. It was discovered later that evaporating the reaction mixture at about 80° under vacuum produces XIIa directly in better yield than first converting to hemihydrate and dehydrating to the diketone as shown in Scheme 3. Absorption and NMR spectra (not shown) and mass balance verifies the structure of the hemihydrate



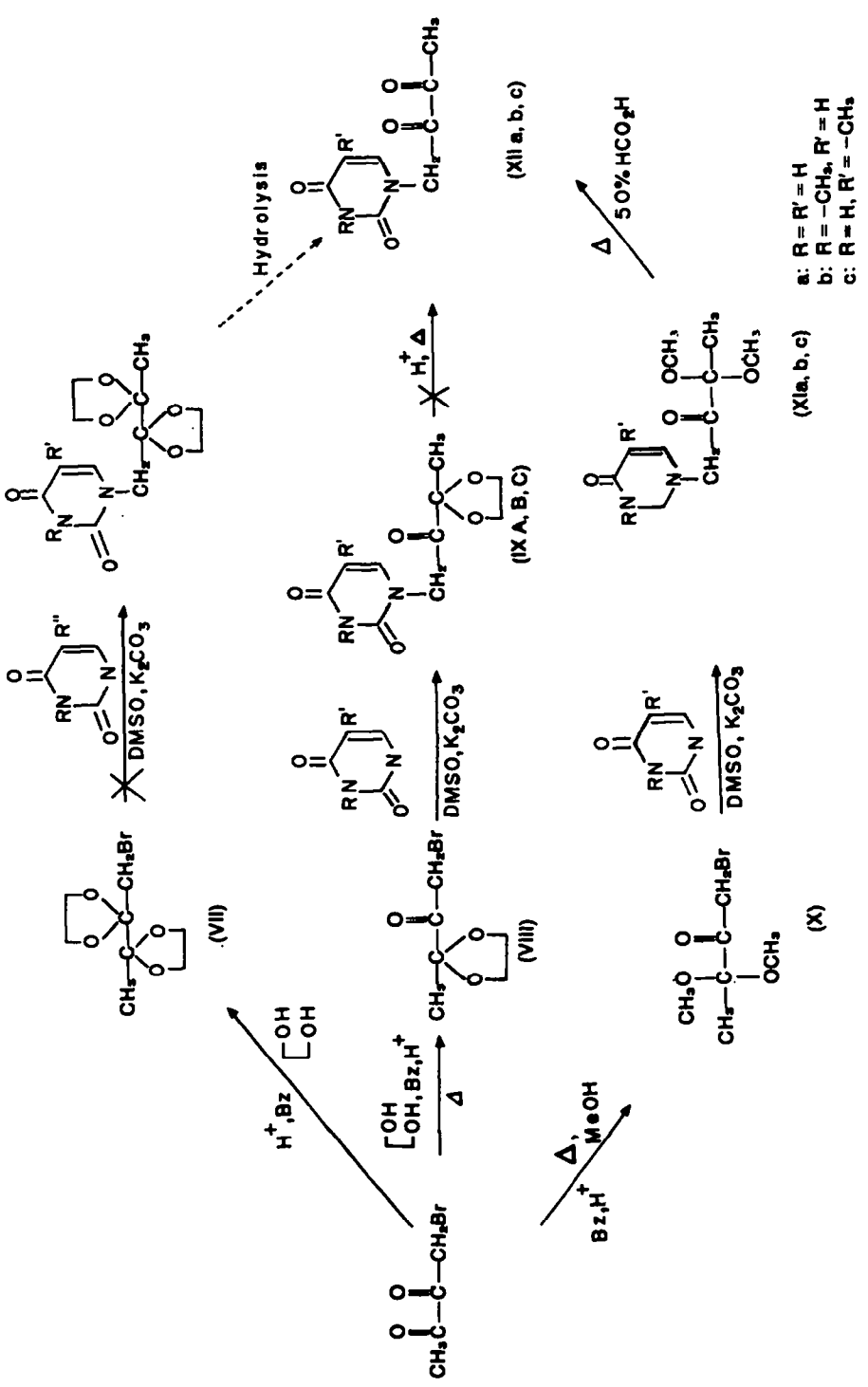
Scheme 1

(XIIIa). Absorption of the methylene proton (adjacent to the carbonyl) in the NMR of XIIa and XIIIa is quite different in dimethylsulfoxide-d and water (Scheme 3). In deuterium oxide the values of chemical shift of the methylene protons are the same (δ 3.95) in both XIIa and XIIIa, which unequivocally suggested that the colorless XIIIa is a hemihydrate.

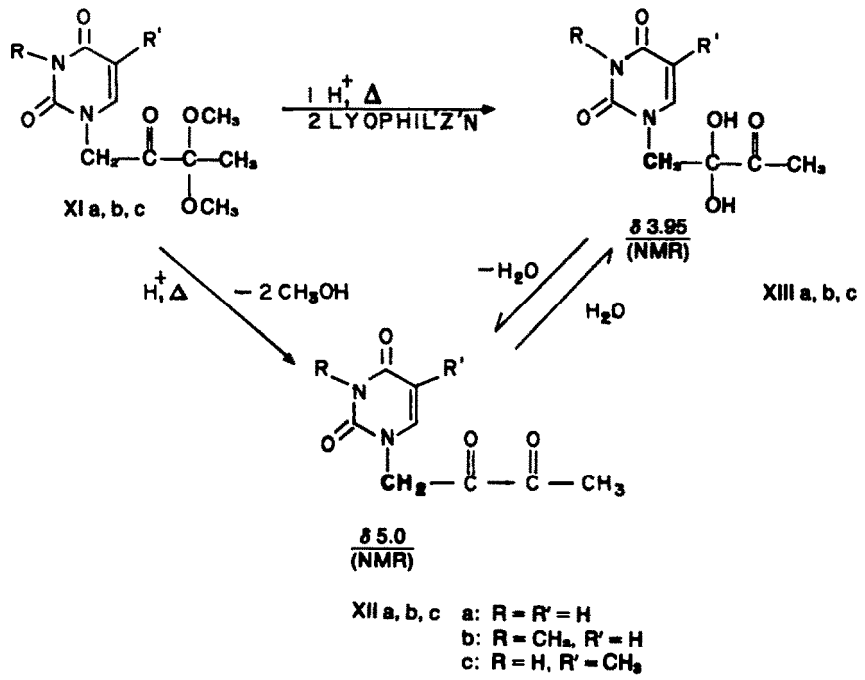
Absorption spectra. The characteristic feature of the α-diketone absorption is the presence of an absorption band in the visible wavelength region where pyrimidines or small conjugated π-systems do not absorb. In Fig. 1 the absorption spectrum of 1-(2,3-dioxopentyl)uracil (V, UPD) is compared to those of its constituent chromophores, 1-ethyl uracil and 2,3-pentanedione (PD). The intense absorption peak at 260 nm of UPD is mainly the uracil absorption and a much weaker absorption band at longer wavelength (*ca.* 410 nm) must be the absorption of the pentanedione moiety. Our primary concern was to know whether the dicarbonyl chromophore was intact in the bichromophoric system and the spectroscopic characters of the isolated diketone were retained. Over all it appears there is no significant modification in the absorption of the individual chromophores of UPD although a slight difference in molar extinction coefficients at the visible absorption maximum is noticed (Table 1). The pyrimidine substituted butanediones (XIIa–c) also exhibit two distinctive absorption bands similar to those of UPD. However, these compounds have more intense visible absorption than the biacetyl as indicated by the data given in Table 1; the color of crystals of XIIa–c are yellow-green while that of UPD is nearly colorless. In addition to the two bands, another weak absorption at about 300–310 nm appears as a shoulder on the uracil band and has a molar extinction coefficient in the range of 350–450 ($M^{-1}\,cm^{-1}$) (Fig. 2). This absorption did not exist in either uracil or biacetyl absorption spectra. The

Table 1. Absorption maximum and molar extinction coefficient

	Absorption maximum (nm)	Molar extinction coefficient ($m^{-1}\,cm^{-1}$)
1-Ethyluracil	267	9.850
2,3-Butadedione	274	18.1 ± 2
	415	20.0 ± 2
2,3-Pentanedione	272	21 ± 2
	416	18 ± 2
UBD (XIIa)	261	10.200
	410	31.5 ± 4
XIIb	260	9.500
	410	36.8 ± 3
XIIc	265	10.700
	410	38.3 ± 4
UPD (V)	260	9.200
	410	22 ± 2


Hydrolysis
(XII a, b, c)
(IX A, B, C)
(VII)
(VIII)
(X)
(XIa, b, c)
DMSO, K2CO3
H+, Bz
H+, Δ
Δ 50% HCO2H
a: R = R' = H
b: R = -CH3, R' = H
c: R = H, R' = -CH3

Scheme 2



Scheme 3

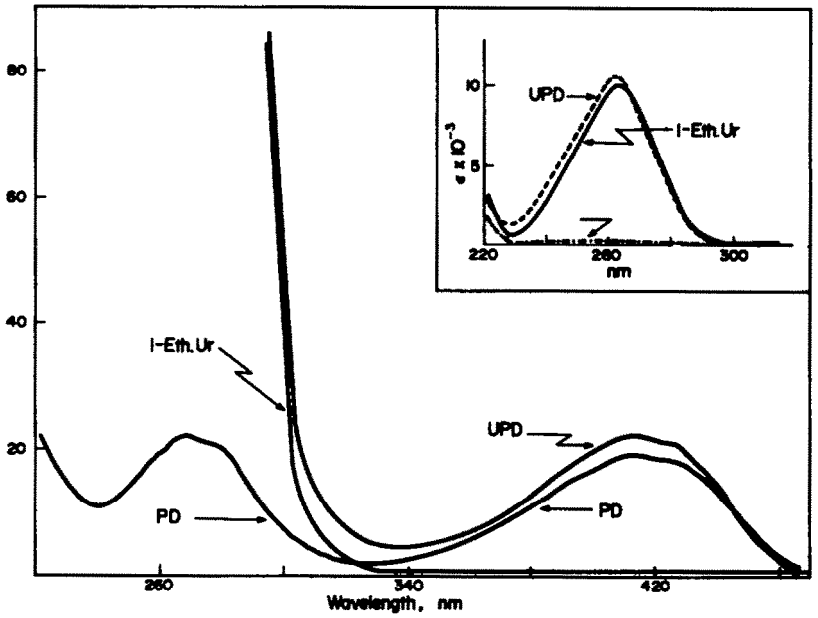


Fig. 1. Absorption spectra. Uracil-pentanedione, 1-ethyl-uracil and 2,3-pentanedione. Upper right corner: absorption spectra in UV region; an expanded scale.

probable existence of an enolic form of uracil[18] cannot be the source of the new absorption band because a similar absorption band is observed in 3-methyluracil-diketone (XIIb) which cannot exist in the enolic form. The low molar extinction coefficient indicates the absorption is probably due to an n,π^* type transition. In polar solvents, this band shifted toward blue and became buried under the intense uracil absorption. Because of the n,π^* nature, probably the diketone is the main source of this new absorption. The difference in the absorption spectra of biacetyl and uracil butanedione suggests that coupling the pyrimidines to the biacetyl has induced a moderate perturbation in the original diketone chromophore. In aqueous or methanolic solution, the visible absorption bands of the bichromophoric compounds, UPD and the butanediones (XIIa–c), are significantly diminished due to the hemihydrate or the hemiketal formation on the diketone moiety. This phenomenon parallels the behavior of biacetyl or PD in protic solvents.[15,16]

Such reactions of the diketone with water or methanol can be easily monitored by observing the change in NMR spectra from aprotic (i.e. acetonitrile) to a protic solvent.

Fluorescence and phosphorescence. For a survey of

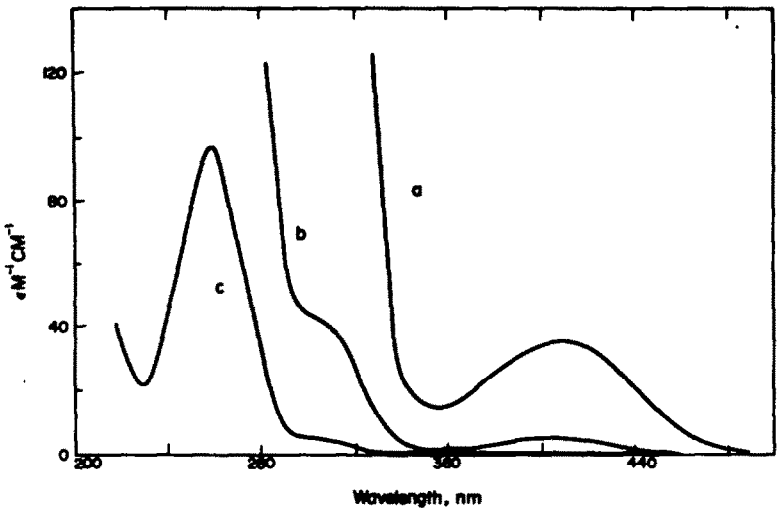


Fig. 2. Absorption spectra of uracil-butanedione (UBD). (a) 5.4×10^{-3} M. (b) 5.4×10^{-4} M. (c) 5.4×10^{-5} M.

the emission of the pyrimidine-diketones, two wavelength regions of absorption are available for excitation, one at 260 nm and the other at 410 nm. With the visible band excitation, the diketone moiety can be selectively excited. Direct excitation of the diketone group in the bichromophoric compounds would enable us to compare the emission of bichromophoric diketones to that of biacetyl or PD. All the bichromophoric compounds exhibit fluorescence near 465 nm where the biacetyl fluorescence maximum is. Fluorescence quantum yields are in the same order of magnitude as biacetyl (10^{-3}) (Table 2). Uracil-pentanedione exhibits both

Table 2. Fluorescence quantum yields†

	λ_{max}	ϕh ($\times 10^3$)
2,3-Butanedione (BD)	460	2.5
2,3-Pentanedione (PD)	460	2.6
UBD (XIIa)	458	2.2
UPD (V)	462	3.9
XXIb	465	1.0
XIIc	460	0.85

†Relative Fluorescence Quantum Yield based on reported biacetyl (BD) fluorescence quantum yield in organic solvents.[19]

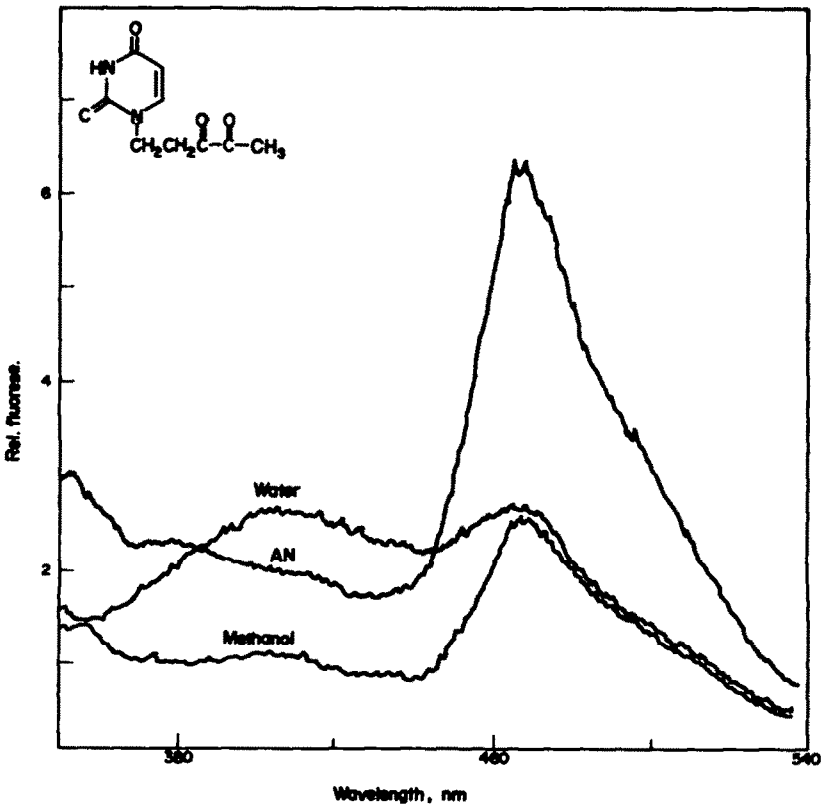


Fig. 3. Fluorescence spectra of uracil-pentanedione (UPD) in water, acetonitrile and methanol.

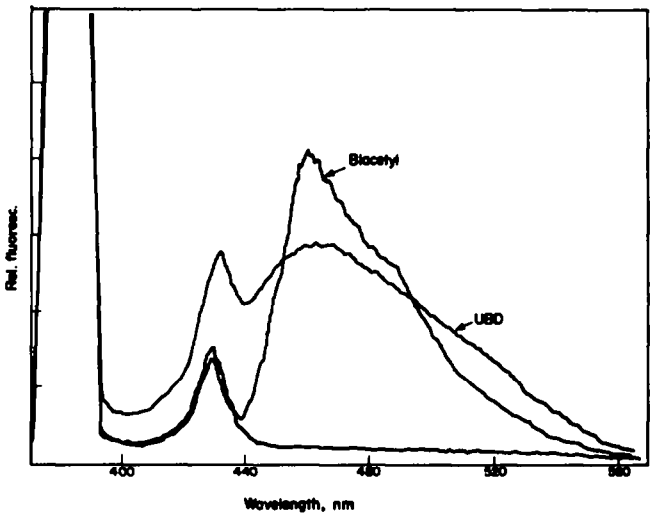


Fig. 4. Fluorescence spectra of uracil-butanedione (UBD) and biacetyl.

fluorescence and phosphorescence in acetonitrile with similar spectral distribution as PD emission spectra. However, the phosphorescence is weaker than in the PD case with a phosphorescence/fluorescence ratio of 0.25, compared to a ratio of 1.0 for PD. Contrary to UPD, fluorescence of the pyrimidine substituted butanediones (XIIa–c) is generally weaker with a broader emission band than that of biacetyl (Fig. 4). No phosphorescence is observable in these bichromophoric butanediones even with rigorous degassing. This is in a marked contrast to biacetyl emission which displays a strong phosphorescence.[19] Such a difference was also evidenced in absorption spectra discussed in the preceding section. This indicates that substitution of pyrimidines α to the diketone functional group has modified the spectral property of the diketone to a greater extent than substitution in the β position as in the case of UPD.

The reaction of the diketones with protic solvents which have been discussed earlier is also clearly manifested in their fluorescence spectra as shown in Fig. 3. In water or methanol, a new fluorescence maximum of the hemihydrate or hemiketal appears at about 420 nm, and at the same time the original diketone fluorescence at 465 nm (maximum) is considerably diminished. This series of bichromophoric diketones behaves identically to biacetyl in protic solvents with respect to hydrate or hemiketal formation which has been previously reported.[15] It should be noted that in aqueous or alcoholic solutions of bichromophoric diketones, the side chain diketone exists in equilibrium with the hemihydrate or hemiketal form. This equilibrium is affected by temperature and mass action. The phosphorescence of UPD is nearly nonexistent in water partially because of the hydrate formation. Thus the utility of pyrimidine substituted diketones for excitation energy transfer in aqueous solution is accordingly diminished compared to that in aprotic solvents; however, such limitations may be overcome at least in part by extrapolation of information obtainable in mixed aqueous solvents to pure water.

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REFERENCES

[1]J. G. Burr, W. A. Summers and Y. J. Lee, *J. Am. Chem. Soc.* **97**, 245 (1975).
[2]W. A. Summers, Y. J. Lee and J. G. Burr, *J. Org. Chem.* **40**, 1559 (1975).
[3]Y. J. Lee, W. A. Summers and J. G. Burr, *J. Am. Chem. Soc.* **99**, 7679 (1978).
[4]M. P. Doerner, *U.S. Pat.* 2,821,555 (1958).
[5]I. I. Grandberg and T. I. Zuyanova, *Khim. Geterosikl. Edin* 875 (1968).
[6]W. S. Johnson, E. R. Rogier, J. Szmuszkovicz, H. I. Hadler, J. Ackerman, B. K. Bhattacharyya, B. M. Bloom, L. Stalmann, R. A. Clement, B. Bannister and H. Wynberg, *J. Am. Chem. Soc.* **78**, 6300 (1956).
[7]B. R. Baker, G. D. Jackson and G. B. Chedda, *J. Pharm. Sci.* **54**, 1617 (1965).
[8]D. J. Brown, J. Eisinger and N. J. Leonard, *J. Am. Chem. Soc.* **90**, 7302 (1968).
[9]O. Manasse, *Ber.* **21**, 2176 (1888).
[10]M. Hollemann, *Rec. Trav. Chem.* **10**, 214 (1895).
[11]H. Tuhoffen, H. Pommer and F. Bohlman, *Ber.* **81**, 507 (1948).
[12]E. R. Geazier, *J. Org. Chem.* **27**, 2937 (1962).
[13]N. Korhblum and H. W. Frazier, *J. Am. Chem. Soc.* **88**, 865 (1966).
[14]H. Gopal and A. J. Gordon, *Tetrahedron Letters* **31**, 2941 (1971).
[15]Y. J. Lee and J. G. Burr, *Chem. Phys. Letters* **43**, 146 (1976).
[16]P. Greenzaid, Z. Luz and D. Samuel, *J. Am. Chem. Soc.* **89**, 749 (1967).
[17]R. P. Bell, *Advances in Physical Organic Chemistry* (Edited by V. Gold), Vol. 4. Academic Press, New York (1966).
[18]M. Daniels, *Proc. Nat. Acad. Sci.*, U.S.A. **69**, 2488 (1972).
[19]M. Almgren, *Mol. Photochem.* **4**(3), 327 (1972).