SYNTHESIS AND CHEMILUMINESCENCE OF 1,3-DISUBSTITUTED 4-HYDROXYPYRAZOLO[4',3':5,6]PYRIDO[2,3-*d*]PYRIDAZINE-5,8(6*H*,7*H*)-DIONES

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Abstract-----Reactions of 1,3-disubstituted 5-aminopyrazole-4-carboxylate derivatives (**1a-c**) with dimethyl acetylenedicarboxylate gave the corresponding dimethyl 1,3-disubstituted 4-hydroxypyrazolo[3,4-*b*]pyridine-5,6-dicarboxylates (**2a-c**) which reacted with hydrazine hydrate to give 1,3-disubstituted 4-hydroxy-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-diones (**3a-d**). These tricyclic pyridazine derivatives were alternatively synthesized from 4-hydroxypyrrolo[3,4-*e*]pyrazolo[3,4-*b*]pyridine-5,7-diones (**7a-c**) prepared by reactions of 5-aminopyrazoles (**1e-g**) with 1-methyl-3-methylthio-4-methoxycarbonylmaleimide (**5**) followed by Gould-Jacacobs reaction. These tricyclic pyridazine derivatives were evaluated for chemiluminescence. 4-Hydroxy-3-methylthio-1-phenyl-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-dione (**3d**) showed the greatest chemiluminescence intensity in the presence of H_2O_2 and peroxidase in a solution of phosphate buffer at pH 10.

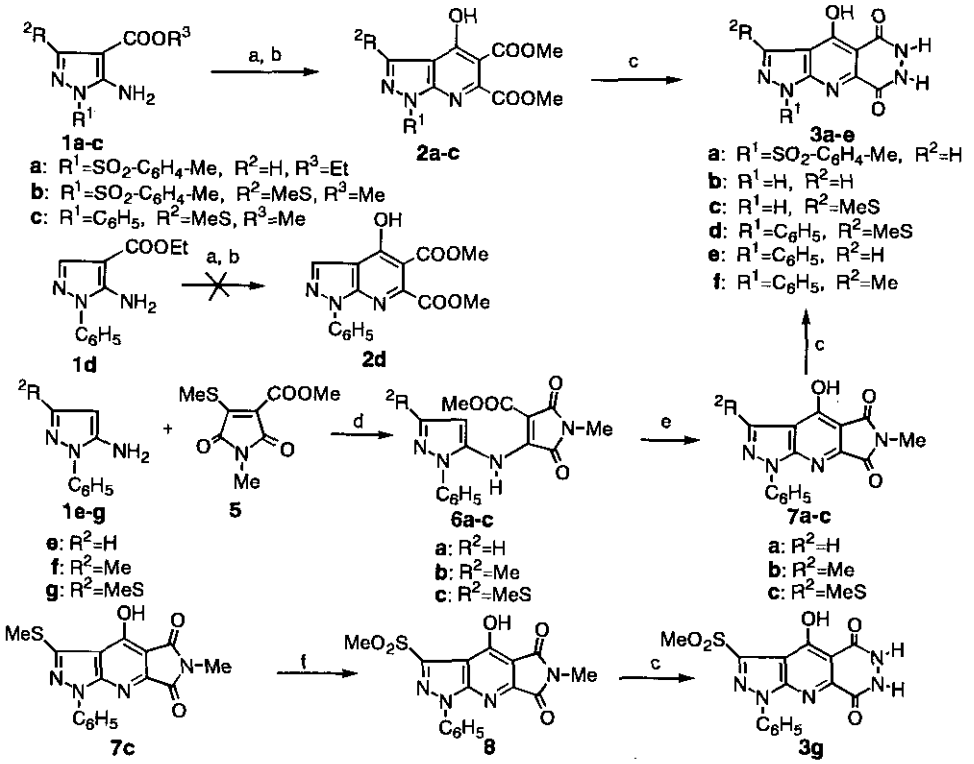
Since the discovery of luminol by Albrecht in 1928, the analytical usefulness of its chemiluminescence (CL) has been amply demostrated.[1] CL of luminol has been developed not only for metal analysis of Co(II), Cu(II), Ni(II), Fe(II), and others but also been found ideal for clinical chemistry such as quantitative determination of blood glucose using enzyme induced CL of luminol.[2] 4-Amino-*1H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(*6H,7H*)-diones were previously shown more efficient than luminol in light production.[3] The authors have directed attention to the chemiluminescence of the compounds bearing a hydroxy group instead of an amino group. In some cases, the hydroxy group was shown capable of affecting fluorescence and light production.[4] No study on CL of poly cyclic pyridazinediones or related compounds bearing a hydroxy group has been conducted to date. Details regarding to CL of 1,3-disubstituted 4-hydroxypyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(*6H,7H*)-diones are presented in this paper.

The chemiluminescent compounds, 4-aminopyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(*6H,7H*)-diones were prepared from dimethyl 4-aminopyrazolo[3,4-*b*]pyridine-5,6-dicarboxylates obtained by reactions of 5-aminopyrazole-4-carbonitriles with dimethyl acetylenedicarboxylate (DMAD) in the presence of potassium carbonate as a base in DMSO.[5] This type of process was considered well applicable to a synthesis of key compounds, dimethyl 4-hydroxypyrazolo[3,4-*b*]pyridine-5,6-dicarboxylates. The reactions of ethyl 5-amino-1-*p*-toluenesulfonylpyrazole-4-carboxylate (**1a**)[6] with DMAD in the presence of potassium carbonate as a base in DMSO gave the corresponding dimethyl 1-*p*-toluenesulfonyl-4-

hydroxypyrazolo[3,4-*b*]pyridine-5,6-dicarboxylate (**2a**) in 61% yield. Similarly, the 3-methylthio derivative (**2b**) was prepared by the reaction of **1b**[7] with DMAD in 48% yield. While the reaction of **1c**[8] with DMAD gave the desired product (**2c**) in only 3% yield, the desired product (**2d**) could not be obtained at all from the reaction of ethyl 5-amino-1-phenylpyrazole-4-carboxylate (**1d**)[9] with DMAD under same conditions.[10] 3-Unsubstituted 1-aryl compounds were not obtained by the this method.

To obtain the 3-unsubstituted 4-hydroxpyrazolopyridopyridazine-5,8-diones and increse the yield of **2d**, an alternative method of synthesis for the pyrazolopyridopyridazine derivatives had to be established. Reaction of 5-amino-1-phenylpyrazole (**1e**)[11] with 1-methyl-3-methylthio-4-methoxycarbonylmaleimide (**5**)[12] under refluxing in methanol gave the corresponding displacement product of the methylthio group (**6a**) in **5** in 82% yield. The cyclization of **6a** by heating in diphenyl ether afforded 4-hydroxy-3-methyl-1-phenylpyrrolo[3,4-*e*]pyrazolo[3,4-*b*]pyridine-5,7-dione (**7a**) in 93% yield. In a similar manner, other compounds (**7b, c**) were readily obtained from the corresponding 5-aminopyrazole derivatives (**1f, g**[13]) in 65% and 73% (from **1f, g**) yields, respectively.

Dimethyl 4-hydroxy-3-methylthio-1-phenylpyrazolo[3,4-*b*]pyridine-5,6-dicarboxylate (**2a**) was refluxed with excess hydrazine hydrate in ethanol followed by removal of ethanol by distillation to give **3a** in 87% yield. When hydrazine hydrate was used in large excess, desulfonylation simultaneously occurred to give **3b** in 67% yield. In a similar manner, 4-hydroxy-3-methylthiopyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8-(*6H,7H*)-dione (**3c**) was obtained from **2b** in 62% yield. 1,3-Disubstituted compound (**3d**) was



a: DMAD, K_2CO_3 in DMSO, room temperature, 20 h; b:10% HCl; c: $H_2NNH_2{\cdot}H_2O$, reflux in MeOH; d: reflux in MeOH, 30 min-1 h; e: reflux in diphenyl ether, 30 min; f: *m*-chloroperbenzoic acid in $CHCl_3$.

Scheme 1

obtained from **2c** in 91% yield. Compounds(**3d**, **e** and **f**)were synthesized by reactions of **7a-c** with *hydrazine hydrate under refluxing in methanol* in 78, 82, and 76% yields, respectively.[14]

The oxidation of **7c** with excess MCPBA (*m*-chloroperbenzoic acid) in chloroform at room temperature gave the desired sulfonyl product (**8**) in good yield. The 3-methylsulfonyl derivative(**3g**)was obtained from **8** in 86% yield.

The CL intensity in this pyrazolopyridopyridazine series is shown in Table 1.[15] 4-Hydroxy-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-diones (**3d-g**) showed nearly the same or somewhat stronger light intensity than luminol at pH 8.0. 1-Unsubstituted compounds(**3b**, **c**)were not shown CL at pH 8.0. Compounds(**3a-g**)also showed CL similar to luminol at pH 10.0. The aryl group at the 1-position is very important for CL production. The light emission of methylsulfonyl compound (**3g**) did not decrease. These compounds showed increased light intensity with rise in pH, as noted also for luminol. The use of HRP(horseradish peroxidase) as POD(peroxidase) gave satisfactory results for CL production.

Table 1. Chemiluminescence Intensity of Hydoxy-polycyclic Pyridazinedione Derivatives

Compound	CL(c.p.10sec)[a] pH 10 pH 8	Compound	CL(c.p.10sec)[a] pH 10 pH 8
3a	1.0×10^5 9.6×10^2	3e	1.6×10^6 9.0×10^4
3b	1.7×10^5 0	3f	1.2×10^5 8.5×10^3
3c	7.2×10^4 0	3g	9.9×10^4 1.5×10^4
3d	9.5×10^6 2.6×10^3	Luminol	1.5×10^7 8.5×10^3

a) Counts per 10 sec. (Their values were subtracted from each back ground.)

A reaction solution containes 10 mmol/l phosphate buffer pH 8.0, 0.5 ml/l Triton X-100, 2.5×10^{-7} mol/l test compound, and 2500 U/l HRP (The each test compound was prepared to obtain concentration of 1.5×10^{-5} mol/l in DMSO). The solution (3 ml of vol) was transfered to a Borosilicate glass tube (12x75 mm) and immediately placed in water bath (37°C) for 10 min. At the end of incubation period, the sample tube to be counted was incorporated into a luminometer. Photons were counted for 10 seconds after addition of 0.3 ml of 1.1×10^{-5} mol/l H_2O_2 (1.0×10^{-6} mol/l as final concentration) and 0.3 ml of 0.2 mol/l glycine buffer pH 8.0 or pH 10.

In conclusion, 4-hydroxypyrazolopyridopyridazine-dione derivatives are chemiluminescent compounds in strong basic solution (pH 8.0 or pH 10). Maleimide (**5**) should prove useful for obtaining heterocycles containning the pyrrole ring.

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6. **1a**: This compound was prepared by reaction of ethyl ethoxymethylenecyanoacetate with *p*-toluenesulfonylhydrazide under reflux in methanol in 77% yield, mp 151-152°C.
7. **1b**: This compound was prepared from methyl bis(methylthio)methylenecyanoacetate and *p*-toluenesulfonylhydrazide in the same manner as the preparation for **1a** in 73% yield. mp 155-156°C, colorless prisms.
8. **1c**: R. Gompper and W. Topfl, *Chem. Ber.*, 1962, 95, 2881.
9. **1d**: L. Bauer and C. S. Mahajanshetti, *J. Heterocyl. Chem.*, 1967, 4, 325.
10. Unknown compound: colorless prisms, mp 153-155°C. Ir(KBr) ν cm^{-1}: 1750, 1710(CO), 1608, 1210, 1165. ^{1}H-Nmr(CDCl$_3$)δ: 1.33(3H, t, J=7.0, OCH$_2$-CH$_3$), 3.65(6H, s, OMe), 3.66(6H, s, OMe), 4.33(2H, q, J=7.0 Hz, OCH$_2$-CH$_3$),5.51(2H, s, -CH$_2$-COOMe), 7.27-7.69(5H, m, phenyl-H), 8.09(1H, s, N=C-H). FABms:516(M^++1). *cf.* H. Matsunaga, M. Sonoda, Y. Tomioka, and M. Yamazaki, *Chem. Pharm. Bull.*, 1984, 32, 2596.
11. Commercially available from Tokyo Kasei.
12. Compound (5) was obtained by the hydrolysis of methyl 2-methoxyimino-3-methylthio-5-oxo-pyrroline-4-carboxylate with *c* HCl in 67% yield. mp 120-121°C. Ir(KBr) ν cm^{-1}: 1745, 1710, 1685(CO), 1530, 1440, 1200. ^{1}H-Nmr(CDCl$_3$)δ: 2.88(3H, s, SMe), 3.05(6H, s, NMe), 3.90(6H, s, OMe). *cf.* M. Sone, Y. Tominaga, R. Natsuki, Y. Matsuda, and G. Kobayashi, *Chem. Pharm. Bull.*, 1973, 21, 1667.
13. Compound (**1g**) was prepared by the hydrolysis of **1c** with 10% NaOH-MeOH solution followed by decarboxylation under heating at ca. 300°C; colorless prisms, mp 114-115°C. ^{1}H-Nmr(CDCl$_3$)δ: 2.51(3H, s, SMe), 3.78(2H, br s, NH$_2$), 5.60(1H, s, 4-H), 7.30-7.56(5H, m, phenyl-H).
14. Satisfactory spectral (ir, uv, ^{1}H-nmr, and ms) data were obtained for all new compounds in this work. **3d**: mp 360°C. Ir(KBr) ν cm^{-1}: 3320(OH), 3200-2800(br, NH or OH), 1700, 1620(CO), 1580, 1500, 1380. Uv λ max nm: 227, 254, 261, 306, 335, 367. Nmr(DMSO-d$_6$)δ: 2.50(3H, s, SMe), 7.30-7.64(3H, m, phenyl-H), 8.18-8.38(2H, m, phenyl-H). Ms m/z: 342(M^++1, 24), 341(M^+, 100), 308(46), 77(10).
15. CL intensity was measured with a Magic Lite Analyzer of CIBA-CORNING. CL spectrum was measured with the spectrofluorometer, Shimadzu RF-510.
16. Maximum wavelength in chemiluminescent spectrum of **3d** was about 420 nm in the presence of 0.01 ml 4N NaOH solution and 0.01 mg/ml(290 μM) **3d** in dimethyl sulfoxide.

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