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Studies on Stable Diazoalkanes as Potential Fluorogenic Reagents. II.[1] Ring-Fused 4-Diazomethylcoumarins

KEIICHI ITO* and JUNKO MARUYAMA

Hokkaido Institute of Pharmaceutical Sciences, Katsuraoka-cho, Otaru-shi 047–02, Japan

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Several ring-fused 4-diazomethylcoumarins **4a**—**f** were prepared as stable diazoalkanes, and their usefulness for the fluorescent labeling of acidic substances was examined. The most easily accessible product, 4-diazomethyl-2*H*-naphtho[1,2-*b*]pyran-2-one **4a**, reacted readily with carboxylic acids in the presence of silica gel catalyst, and also with alcohols in the presence of fluoroboric acid catalyst, to produce fluorescent esters and ethers in good yields.

Keywords——stable diazoalkane; benzo-fused 4-diazomethylcoumarin; carboxylic acid fluorescent esterification; fluorescence-labeled alcohol; fluorescence quantum yield

7-Substituted 4-diazomethylcoumarins, recently reported[1,2] from this laboratory, are versatile fluorescent labeling reagents for acids and alcohols for high-performance liquid chromatographic and other analytical purposes.[1-3] In this paper, we report on the preparation and properties of some ring-fused analogs of 4-diazomethylcoumarin **4a**—**e** and the analogous compound **4f** as readily available diazoalkanes with high stability which are potentially useful as tricyclic fluorogenic reagents.

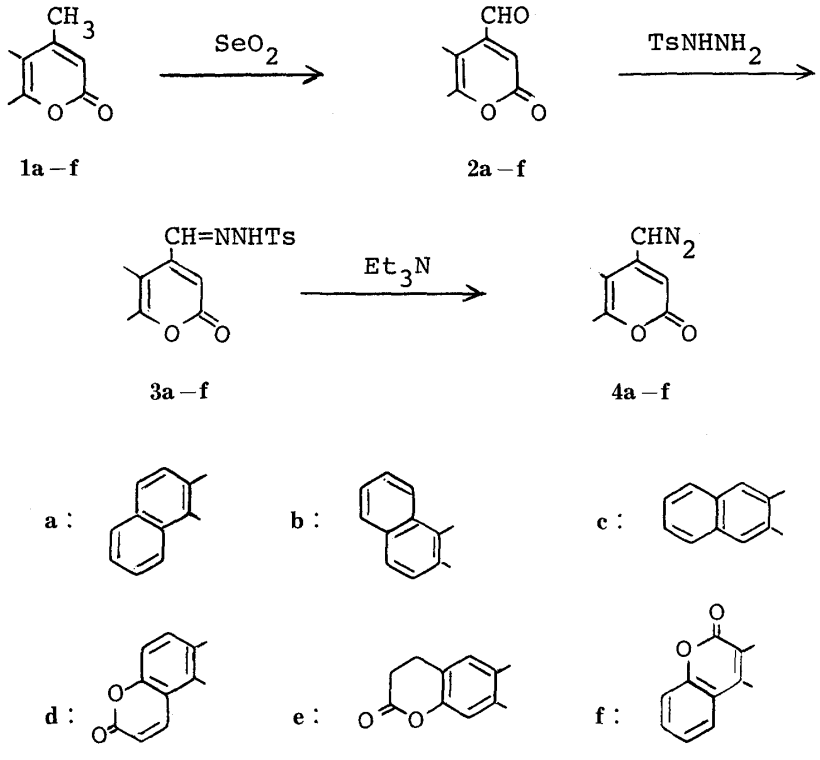


Chart 1

TABLE I. Reactions[a] 1→2(A), 2→3(B) and 3→4(C)

Starting material No.	Reaction A		Reaction B		Reaction C		Final product No.
	Reaction time (h)	Yield (%)	Reaction time (h)	Yield (%)	Reaction time (h)	Yield (%)	
1a	10	82	18	80	2	92	**4a**
1b	10	82	4.5	96	1.5	92	**4b**
1c	28	65	10	88	1.5	81	**4c**
1d	43	71[b]	48	68[b]	4	98	**4d**
1e	23	74	24	66[b]	3[c]	71	**4e**
1f	10	77	1[d]	59	1.5	95	**4f**

a) See the experimental section. *b*) Yield of crude product. *c*) Reaction carried out in $CHCl_3$ at room temperature. *d*) Reaction carried out in refluxing EtOH.

TABLE II. Fused 4-Diazomethylcoumarins **4**

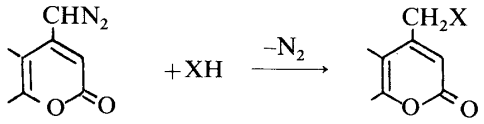
No.	Appearance (Recrystn. solvent)	Formula (m/e M^+)	Analysis (%) Calcd (Found)			^{1}H-NMR (DMSO-d_6) δ ppm		IR $\nu_{CHN_2}^{KBr}$ cm^{-1}	UV λ_{max}^{EtOH} nm (log ε)
			C	H	N	CHN_2 (1H, s)	C^3-H (1H, s)		
4a	Yellow needles[a] (THF)	$C_{14}H_8N_2O_2$ (236)	71.18 (71.29	3.41 3.33	11.86 11.83)	6.01	6.69	2086	259 (4.47), 296 (4.21), 308.5 (4.43), 331 (4.12), 345.5 (4.21)
4b	Yellow prisms[b]	$C_{14}H_8N_2O_2$ (236)	71.18 (70.90	3.41 3.31	11.86 11.70)	6.00	6.59[d]	2088	227 (4.75), 276 (3.99), 298 (4.03), 348 (4.18)
4c	Yellow needles[a] ($CHCl_3$)	$C_{14}H_8N_2O_2$ (236)	71.18 (70.88	3.41 3.25	11.86 11.57)	5.99	6.68	2084	224.5 (4.69), 262.5 (4.58), 321 (4.28), 349 (4.08)
4d	Yellow needles[a] ($CHCl_3$)	$C_{13}H_6N_2O_4$ (254)	61.42 (61.68	2.38 2.39	11.02 10.72)	5.95	6.62	2094	252.5 (4.24), 285 (4.41), 305 (4.38)
4e	Yellow plates[a] (CH_3CN)	$C_{13}H_8N_2O_4$ (256)	60.94 (60.98	3.15 3.10	10.93 11.11)	5.86	6.50	2072	250 (4.10), 258 (4.09), 291 (3.95), 323 (4.21)
4f	Yellow plates[c] (THF)	$C_{13}H_6N_2O_4$ (254)	61.42 (61.52	2.38 2.30	11.02 10.98)	5.90	6.98	2084	227 (4.24), 265 (3.83), 297.5 (4.41), 320.5 (4.31), 335 (4.21)

a) mp >280 °C. *b*) mp 110—112 °C (dec.) without recrystallization. *c*) mp 169—172 °C. *d*) C^2-H.

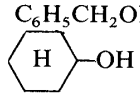
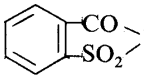
The new diazoalkanes **4a**—**f** can be easily prepared in three steps starting from the corresponding fused 4-methylcoumarins **1a**—**f**[4] by a procedure similar to that reported previously,[1] *i.e.*, selenium dioxide oxidation of **1** in refluxing xylene, conversion of the resulting 4-formyl derivatives **2** into tosylhydrazones **3**, and Bamford–Stevens reaction[5] of **3** by the use of triethylamine in methanol, as shown in Chart 1 and Table I. However, **4e** was obtained from **3e** by the reaction in anhydrous chloroform suspension, since the reaction in methanol resulted in triethylamine-catalyzed methanolysis of the dihydropyrone ring of **4e** even at room temperature.

All the diazo compounds prepared are yellow crystals of high melting point (except **4b** and **4f**), exhibiting characteristic infrared (IR) and proton nuclear magnetic resonance (^{1}H-

TABLE III. Reactions of **4** with Acidic Substances XH



4a—f **5a—f, 6a—14a**

Diazo reagent No.	XH	Reaction conditions			Product	Yield (%)
		Catalyst	Temp. (°C)	Time (h)		
4a	CH_3COOH	SiO_2	Reflux	2	**5a**	92
4b	CH_3COOH	SiO_2	40—45	2	**5b**	69[a]
4c	CH_3COOH	SiO_2	Reflux	1.5	**5c**	89
4d	CH_3COOH	SiO_2	Reflux	8	**5d**	91
4e	CH_3COOH	SiO_2	Reflux	3	**5e**	95
4f	CH_3COOH	SiO_2	Reflux	1.5	**5f**	90
4a	n-$C_{15}H_{31}COOH$	SiO_2	Reflux	4	**6a**	81
4a	C_6H_5COOH	SiO_2	Reflux	3	**7a**	91
4a	$C_6H_5CH=CHCOOH$	SiO_2	Reflux	5	**8a**	87
4a	CH_3SO_3H	None	RT[b]	1	**9a**	95
4a	p-$CH_3C_6H_4SO_3H$	None	RT	0.5	**10a**	95
4a	C_2H_5OH	HBF_4	RT	0.3	**11a**	91
4a	$C_6H_5CH_2OH$	HBF_4	RT	15	**12a**	71
4a		HBF_4	RT	24	**13a**	57
4a		None	RT	24	**14a**	45

a) An unidentified product was obtained simultaneously. *b*) RT: room temperature.

NMR) spectra, as shown in Table II. No decomposition has been observed after storage of the crystals for more than a year at room temperature. Even in a refluxing solvent such as chloroform or tetrahydrofuran, no significant change was observed except in the case of **4b**, which is exceptionally labile in warm solvent. Furthermore, **4a—f** have sufficient reactivity toward acidic substances in the presence of a catalyst, as demonstrated by the reactions with carboxylic and sulfonic acids, alcohols and saccharin, resulting in the formation of the corresponding esters and ethers in excellent yields.

As shown in Table III, the reactions with acids proceeded rapidly in chloroform with addition of silica gel catalyst at refulx (for carboxylic acid) or without catalyst at room temperature (for sulfonic acid), whereas the reactions with alcohols occurred only in the presence of fluoroboric acid catalyst. In contrast to the near non-fluorescence of the diazo reagents (fluorescence quantum yield $\Phi=0.05$ for **4a—f**), the obtained ester and ether products, **5a—f** and **6a—14a**, exhibit characteristic fluorescent properties in ethanol, depending on the type and the position of the fused ring and also on the acidic component (see Table IV). While linearly benzo-fused **5c** and the pyrono-fused **5d** and **5f** together with the sulfonic acid derivatives (**9a**, **10a**) fluoresce only weakly, an apparent annellation effect on the fluorescence intensity can be seen for the dihydropyrono-fused **5e** and angularly benzo-fused **5a** and **5b**, when the acetate products **5a—f** are compared with the parent coumarin-4-ylmethyl acetate ($\Phi=0.023$).[1] Thus, among the new diazo compounds, the most easily accessible **4a** appears to be applicable as an extremely stable fluorescent labeling reagent for carboxylic acids and alcohols in the presence of a suitable catalyst.

TABLE IV. Fused Coumarin-4-ylmethyl Esters and Ethers **5**—**14**

No.	mp (°C) (Recrystn. solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	IR ν_{CO}^{KBr} cm^{-1}	UV λ_{max}^{EtOH} nm (log ε)	F (EtOH)[a] λ_{max}^{ex} nm	λ_{max}^{em} nm	Quantum[b] yield
5a	164—166[c] (iso-PrOH)	$C_{16}H_{12}O_4$	71.63 (71.60	4.51 4.40)		1725 1759	265 (4.42), 275 (4.50), 291.5 (3.80), 303.5 (3.89), 316 (3.88), 353 (3.72)	273	444	0.11
5b	201—203[c] (Benzene)	$C_{16}H_{12}O_4$	71.63 (71.49	4.51 4.43)		1717	230 (4.75), 248 (4.17), 317 (3.97), 348 (4.01)	371	431	0.15
5c	200—202[d] (Benzene)	$C_{16}H_{12}O_4$	71.63 (71.51	4.51 4.45)		1729	226 (4.71), 263 (4.46), 273 (4.47), 321 (4.24)	331	487	<0.01
5d	205—208[e] (CH_3CN)	$C_{15}H_{10}O_6$	62.94 (62.93	3.52 3.41)		1724 1747 1761	291 (4.33)	295 378	400	<0.01
5e	197—199[f] (THF)	$C_{15}H_{12}O_6$	62.50 (62.62	4.20 4.30)		1723 1789	283 (3.95), 320 (4.02)	333	413 510	0.54
5f	210—212[g] (Benzene)	$C_{15}H_{12}O_6$	62.94 (62.89	3.52 3.54)		1723 1743	237.5 (3.91), 257 (4.04), 266.5 (4.03), 333 (4.09), 345 (4.14)	344	403 420	0.054
6a	90—92[d] (CH_3CN)	$C_{30}H_{40}O_4$	77.55 (77.68	8.68 8.76)		1719	265 (4.41), 275 (4.49), 292 (3.78), 304 (3.87), 317 (3.86), 354 (3.70)	273	444	0.087
7a	184—186[g] (AcOEt)	$C_{21}H_{14}O_4$	76.35 (76.05	4.27 4.30)		1712	265.5 (4.40), 275.5 (4.48), 291.5 (3.78), 304 (3.86), 317 (3.85), 354 (3.70)	274	445	0.13
8a	218—221[d] (CH_3CN)	$C_{23}H_{16}O_4$	77.51 (77.34	4.53 4.57)		1703 1735	266 (4.61), 275.5 (4.69), 316 (3.96), 353 (3.76)	274	445	0.086
9a	178—180[d] (CH_3CN)	$C_{15}H_{12}O_5S$	59.20 (58.94	3.98 4.08)		1705	266 (4.41), 275.5 (4.48), 292 (3.82), 304 (3.90), 314 (3.88), 355 (3.71)	288 317	432	<0.01
10a	196—198[h] (CH_3CN)	$C_{21}H_{16}O_5S$	66.30 (66.54	4.24 4.27)		1723	265.5 (4.38), 275.5 (4.44), 292.5 (3.81), 304.5 (3.88), 317 (3.85), 355 (3.68)	308	431	<0.01
11a	130—131[d] (EtOH)	$C_{16}H_{14}O_3$	75.57 (75.59	5.55 5.63)		1720	265 (4.44), 275 (4.52), 291.5 (3.81), 303.5 (3.89), 316.5 (3.86), 351.5 (3.70)	273	435	0.089
12a	125—127[g] (EtOH)	$C_{21}H_{16}O_3$	79.73 (79.91	5.10 5.05)		1719	265 (4.45), 275 (4.54), 291.5 (3.82), 303.5 (3.92), 316 (3.90), 351.5 (3.74)	273	433	0.070
13a	120—122[i] (Hexane)	$C_{20}H_{20}O_3$	77.90 (77.98	6.54 6.48)		1711	265 (4.46), 275 (4.56), 291.5 (3.83), 303.5 (3.92), 316.5 (3.91), 351 (3.75)	274	432	0.077
14a	>260[d] (DMF)[j]	$C_{21}H_{13}NO_5S$	64.44 (64.65	3.35 3.38	3.58 3.37)	1722	264.5 (4.38), 274.5 (4.47), 290.5 (3.85), 303 (3.85), 315.5 (3.81), 350 (3.61)	304	432	0.10

a) Fluorescence: ex, excitation; em, emission. *b*) Relative to quinine sulfate (0.55). *c*) Yellow prisms. *d*) Pale yellow prisms. *e*) Pale yellow needles. *f*) Prisms. *g*) Pale yellow leaves. *h*) Plates. *i*) Leaves. *j*) DMF: dimethylformamide.

Experimental

All melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. IR spectra were determined using a Hitachi 215 grating spectrophotometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-FX-100 spectrometer (100 MHz). Mass spectra (MS) were taken on a Shimadzu LKB-900B spectrometer. Ultraviolet (UV) spectra were obtained in EtOH with a Hitachi 200—10 spectrophotometer. Fluorescence (F) spectra were measured in non-fluorescent EtOH on a Shimadzu RF-503 spectrofluorometer. Relative fluorescence quantum yields were determined according to the method of Parker and Rees[6] using quinine sulfate in 0.1 N H_2SO_4 as the standard.

TABLE V. Fused 4-Formylcoumarins **2** and Their Tosylhydrazones **3**

No.	mp (°C) (Recrystn. solvent)	Appearance	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR ($CDCl_3$) δ ppm C^3-H (1H, s) CHO (1H, s)
2a	200—203[a] (CH_3CN)	Brown leaves	$C_{14}H_{18}O_3$	74.99 (74.86	3.60 3.74)		6.90 10.15
2b	170—172 (THF)	Yellow needles	$C_{14}H_{18}O_3$	74.99 (75.15	3.60 3.77)		6.71[b] 10.59
2c	228—230 (CH_3CN)	Yellow plates	$C_{14}H_{18}O_3$	74.99 (74.74	3.60 3.70)		6.92 10.18
2e	264—265 (CH_3CN)	Brown prisms	$C_{13}H_8O_5$	63.94 (64.04	3.30 3.47)		6.85 10.05
2f	232—235 (THF)	Brown prisms	$C_{13}H_6O_5$	64.47 (64.18	2.50 2.80)		6.47[c] 10.49[c]
3a	195—198 (dec.) (THF)	Yellow prisms	$C_{21}H_{16}N_2O_4S$	64.27 (64.23	4.11 4.09	7.14 7.02)	
3b	205—207 (dec.) (THF)	Pale yellow needles	$C_{21}H_{16}N_2O_4S$	64.27 (64.42	4.11 4.07	7.14 7.35)	
3c	208—210 (dec.) (THF)	Yellow needles	$C_{21}H_{16}N_2O_4S$	64.27 (63.96	4.11 3.92	7.14 7.25)	
3f	155—158 (THF)	Pale yellow needles	$C_{20}H_{15}N_2O_4S$	58.40 (58.79	3.68 3.29	6.81 7.17)	

a) Lit.[7] mp 200—203 °C. *b*) C^2-H. *c*) Determined in DMSO-d_6.

Preparation of Fused 4-Formylcoumarins 2a—f. 4-Formyl-2*H*-naphtho[1,2-*b*]pyran-2-one (2a)——Pulverized selenium dioxide (9.0 g, 80 mmol) was added to a solution of **1a**[4] (17 g, 80 mmol) dissolved in hot dry xylene (450 ml), and the whole was refluxed with vigorous stirring. After 5 h, further selenium dioxide (9.0 g, 80 mmol) was added all at once and the whole was again refluxed. After being stirred at reflux for a total of 10 h, the reaction mixture was filtered hot to remove black Se. The deep orange filtrate was allowed to stand overnight and the deposited yellow crystals were collected, dried and recrystallized from CH_3CN to give brown leaves of **2a** (14.7 g, 82%), mp 200—202 °C (lit.[7] mp 200—203 °C).

Compounds **2b—f** were obtained from **1b—f**[4] by the same procedure as described for **2a**; yields are shown in Table I. Physical and analytical data for **2a—f** are listed in Table V.

Preparation of Fused 4-Formylcoumarin Tosylhydrazones 3a—f. 4-Formyl-2*H*-naphtho[1,2-*b*]pyran-2-one Tosylhydrazone (3a)——A mixture of **2a** (6.7 g, 30 mmol) and *p*-toluenesulfonylhydrazine (5.6 g, 30 mmol) suspended in EtOH (200 ml) was vigorously stirred at room temperature. After 18 h, the precipitates were collected, washed with EtOH and recrystallized from tetrahydrofuran (THF) to give **3a** as yellow prisms (9.4 g, 80%), mp 195—198 °C (dec.).

Compounds **3b—f** were obtained by the same procedure as described for **3a**; yields are shown in Table I. In the cases of **3d** and **3e**, the crude products were difficult to purify, and were used directly for the next step. Melting points and analytical data for **3a—c** and **3f** are listed in Table V.

Preparation of Fused 4-Diazomethylcoumarins 4a—f. 4-Diazomethyl-2*H*-naphtho[1,2-*b*]pyran-2-one (4a)——Triethylamine (1.0 g, 10 mmol) was added dropwise to a stirred suspension of **3a** (3.9 g, 10 mmol) in MeOH (80 ml) at room temperature. The whole was stirred for 2 h at room temperature, then the precipitates in the reaction mixture were collected, dried and recrystallized from THF to give yellow needles of **4a** (2.2 g, 92%), mp > 280 °C.

Compounds **4b—d** and **4f** were obtained by the same procedure as described for **4a**; yields are shown in Table I, and physical and analytical data are listed in Table II.

Preparation of 4-Diazomethyl-6,7-dihydro-2*H*,8*H*-pyrano[3,2-*g*]benzopyran-2,8-dione (4e)——Triethylamine (0.3 g, 3 mmol) dissolved in anhydrous $CHCl_3$ (2 ml) was added dropwise to a stirred suspension of **3e** (1.24 g, 3 mmol) in anhydrous $CHCl_3$ (8 ml) at room temperature. After 3 h, the reaction mixture was concentrated under reduced pressure, and the residue was added and stirred thoroughly with dry THF (5 ml). The resulting precipitates were collected and dried to give **4e**. Yield, 0.54 g (71%). Physical and analytical data: see Table II.

Methyl 4-Diazomethyl-7-hydroxy-6-coumarinpropionate——The collected precipitates obtained from the reaction mixture of **3e** with triethylamine in MeOH by the same procedure as described for **4a** were extracted several times with hot THF. Evaporation of the solvent from the combined extracts gave the methanolysis product of **4e**, 179 mg (98%). Recrystallization from THF gave yellow leaves, showing mp 156—157 °C (dec.) after drying at 80 °C *in vacuo*. IR ν_{max}^{KBr} cm^{-1}: 2076 (CHN_2), 1730, 1692, 1669 (ester CO). ^{1}H-NMR (DMSO-d_6) δ: 2.52—2.83 (4H, m, CH_2CH_2), 3.60 (3H, s, CH_3O), 5.63 (1H, s, CHN_2), 6.46 (1H, s, C^3-H), 6.71 (1H, s, C^8-H), 7.46 (1H, s, C^5-H). *Anal.* Calcd for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.20; N, 9.72. Found: C, 58.15; H, 4.37; N, 9.70.

Reaction of the product with AcOH at room temperature gave methyl 4-acetoxymethyl-7-hydroxy-6-coumarinpropionate, prisms from AcOEt, mp 194—196 °C. IR ν_{max}^{KBr} cm^{-1}: 1739, 1720, 1706 (ester CO). ^{1}H-NMR (DMSO-d_6) δ: 2.19 (3H, s, CH_3CO), 2.60—2.84 (4H, m, CH_2CH_2), 3.59 (3H, s, CH_3O), 5.29 (2H, s, $ArCH_2O$), 6.18 (1H, s, C^3-H), 6.77 (1H, s, C^8-H), 7.46 (1H, s, C^5-H). *Anal.* Calcd for $C_{16}H_{16}O_7$: C, 60.00; H, 5.04. Found: C, 59.82; H, 5.23.

Reaction of Fused 4-Diazomethylcoumarins 4a—f with Acidic Substances——General Procedure: A mixture of 3 mmol of **4**, 3 mmol of an acidic substance and 800 mg of silica gel (Wakogel C-200) in 20 ml of $CHCl_3$ was vigorously stirred for the period and at the reaction temperature specified in Table III. In the cases of the reactions with alcohols, two drops of 40% HBF_4 were added instead of silica gel; in the cases of sulfonic acids and saccharin, neither silica gel nor HBF_4 was added.

The reaction mixture was filtered, washed well with $CHCl_3$ and concentrated to give the product, which was recrystallized. Yields: see Table III. Physical, spectral and analyses data: see Table IV.

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