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Studies on Stable Diazoalkanes as Potential Fluorogenic Reagents. I. 7-Substituted 4-Diazomethylcoumarins[1)]

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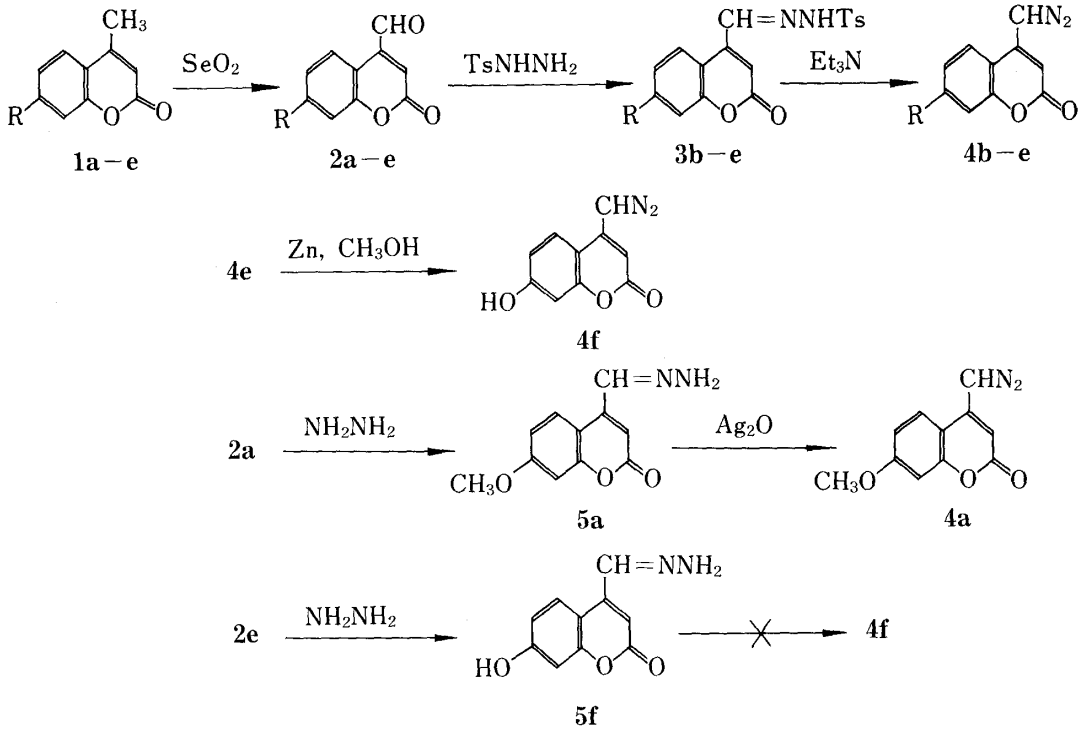
As a new type of stable diazoalkane, 4-diazomethylcoumarins (**4**) bearing various 7-substituents were prepared for potential use in introducing fluorophores into acidic substances. Selenium dioxide oxidation of 4-methylcoumarins to the corresponding coumarin-4-carbaldehydes followed by triethylamine-mediated Bamford–Stevens reaction of their tosylhydrazones readily gave **4** as excellently stable and almost non-fluorescent crystals, exhibiting characteristic diazomethyl ^{1}H- and ^{13}C-nuclear magnetic resonance signals. Labeling reactions of carboxylic acids using **4** in refluxing chloroform in the presence of silica gel catalyst gave excellent yields of fluorescent coumarin-4-ylmethyl esters. In view of their stability, accessibility and fluorogenicity, these compounds (**4**), especially those bearing 7-methoxy and 7-acetyloxy substituents, should be practically useful fluorogenic reagents for carboxylic acids.

Keywords——stable diazoalkane; 4-diazomethylcoumarin; selenium dioxide oxidation; Bamford–Stevens reaction; diazomethyl NMR; silica gel-catalyzed reaction; fluorescent esterification of carboxylic acid; coumarin-4-ylmethyl ester fluorescence

Diazoalkanes, as represented by diazomethane, are well known as versatile reagents for various organic reactions such as esterification of acids, alkylation of alcohols, and so on. Recently the compounds have been shown to be useful as derivatization reagents for acidic substances and they are employed for gas chromatographic, mass spectral (MS) and high-performance liquid chromatographic (HPLC) analyses.[2)] The value of these reagents arises from the fact that diazoalkanes in general react rapidly, with nitrogen as essentially the only by-product. Most conventional gaseous and liquid diazoalkanes must be handled with great care, however, due to their instability, toxicity and potentially explosive nature. Thus, they have to be employed in solutions, prepared from precursors just before use. Attempts have been made to find solid diazoalkanes of adequate stability and reactivity, as well as availability, which can be readily employed as practical reagents. 4-Diazomethyl-*N,N*-dimethylbenzenesulfonamide, prepared by Sekiya *et al.*[3)] and used as an ultraviolet (UV)-labeling reagent for acidic substances,[4)] is perhaps one of quite a few aryldiazomethanes which meet the requirements.

In recent years, fluorescent derivatization has facilitated the development of excellent microdetection procedures in HPLC for chemical, biochemical and clinical purposes.[5)] Starting from the first success in achieving fluorescent labeling by the use of 4-bromomethyl-7-methoxycoumarin,[6)] several compounds reactive toward acidic substances have been reported, including 9,10-diaminoanthracene,[7)] *N,N'*-dialkyl-*O*-(7-methoxycoumarin-4-yl)-methylisoureas,[8)] and 1-bromoacetylpyrene.[9)] Kinoshita *et al.*[10)] recently applied 9-anthryldiazomethane[11)] as a fluorescent label for fatty acids. However, these reagents still suffer from deficiencies in reactivity, stability, work-up procedure and/or availability. In an effort to obtain readily accessible solid diazoalkanes having potentiality to produce fluorophores on reaction with acidic substances, we obtained an excellently stable 4-diazomethyl-7-methoxycoumarin (**4a**), as reported in a preliminary communication.[1)] In this compound, the

reactive diazo function is connected to the coumarin nucleus, whose prominent fluorogenicity has often been utilized for analytical purposes.[12] The same compound was independently reported by Takadate *et al.*[13] as a fluorescent label for alcohols. The present paper describes the results on comparative studies of 4-diazomethylcoumarins of this new type with various 7-substituents, with emphasis on synthesis, spectral properties and reactivity.



a : R=MeO b : R=H c : R=Me d : R=Et_2N e : R=AcO f : R=HO

Chart 1

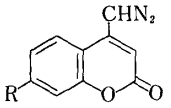
Synthesis

4-Diazomethylcoumarin (**4b**), and several 7-substituted analogs thereof, *i.e.*, methyl (**4c**), diethylamino (**4d**), acetyloxy (**4e**) and hydroxy (**4f**), were selected for comparison with **4a**, because coumarins possessing 7-substituents (especially when they are electron-releasing groups), are most readily accessible and were reported[14] to have enhanced fluorescence. Syntheses were rather straightforward, as shown in Chart 1, and could be achieved in three steps starting from the corresponding 4-methylcoumarins (**1a—e**). Selective oxidation of the 4-methyl substituent to carbaldehyde by the use of selenium dioxide in refluxing xylene, as originally reported by Schiavello *et al.*, [15] was successfully applied to **1a—e** in the yields of 41—88%, despite their comment that the method may be restricted to analogs with 7-alkoxy substituents. Neither further oxidation to carboxylic acid nor oxidation of the 7-methyl group in **1c** was observed. The resulting 4-carbaldehydes (**2b—e**), mostly obtainable in pure crystalline state from the reaction solutions, were converted as usual into their tosylhydrazones (**3b—e**) in 65—96% yields. Bamford–Stevens reaction[16] of **3b—e** was found to be easily carried out under very mild conditions (**3b—e** was treated with an equimolar amount of triethylamine in methanol at room temperature), giving **4b—e** as almost pure precipitates in high yields (80—94%). Treatment of **2e** with excess hydrazine resulted in the formation of 7-hydroxycoumarin-4-carbaldehyde hydrazone (**5f**), the expected intermediate leading to **4f**. Attempted dehydrogenation by using metal oxides, as reported[1] for **4a**, was unsuccessful, however. In contrast, prolonged methanolysis of the 7-acetyloxy substituent of **4e** in the

presence of activated zinc catalyst at room temperature afforded **4f** in 88% yield without any attack at its diazomethyl function. All of the obtained 4-diazomethylcoumarins (**4a—f**) are yellow or brown crystals which can be purified by recrystallization without decomposition, showing no distinct melting or decomposition point except for **4b** and **4e**. Their structures were confirmed by satisfactory microanalyses as well as spectral results as described in the next section.

The most remarkable feature of this type of diazo compound is the extreme stability. The

TABLE I. 7-Substituted 4-Diazomethylcoumarins (**4b—f**)



Compd. No.	R	Total yield[a] (%)	Appearance (Recrystn. solvent)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	MS *m/e* [M$^+$]
4b	H	75	Yellow needles (THF)	156—158 (dec.)	$C_{10}H_6N_2O_2$	64.51 (64.64)	3.25 (3.25)	15.05 (15.05)	186[c]
4c	Me	67	Yellow needles (THF)	[b]	$C_{11}H_8N_2O_2$	65.99 (65.94)	4.03 (3.99)	13.99 (13.85)	200[c]
4d	Et_2N	27	Brown prisms (AcOEt)	[b]	$C_{14}H_{15}N_3O_2$	65.35 (65.27)	5.88 (5.82)	16.33 (16.26)	257[c]
4e	AcO	55	Yellow needles (THF)	165—167 (dec.)	$C_{12}H_8N_2O_4$	59.02 (59.13)	3.30 (3.32)	11.47 (11.32)	244
4f	HO	49	Yellow needles (THF)	[b]	$C_{10}H_6N_2O_3$	59.41 (59.44)	2.99 (2.95)	13.86 (13.63)	202[c]

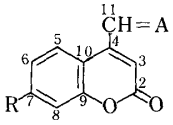
a) Total yield starting from **1b—e**.
b) No distinct melting or decomposition point was observed.
c) Base peak at 70 eV.

TABLE II. IR, UV and Fluorescence Spectral Data for **4b—f**

Compd. No.	IR $\nu_{CHN_2}^{KBr}$ (cm^{-1})	UV λ_{max}^{EtOH} nm (log ε)				F[a] Ex λ_{max} nm	F[a] Em λ_{max} nm
4b	2082	244.5 (4.23)	251 (4.25)	283 (3.93)	320.5 (4.20)		
4c	2078	245 (4.11)	252.5 (4.08)	289 (3.97)	320 (4.21)	385	454
4d	2078	258.5 (4.26)	333 (4.43)	395 (4.28)		370	460
4e	2099	254 (4.32)	319 (4.34)			382	455
4f	2102	242 (4.15)	320 (4.48)			387	468

a) Fluorescence spectrum in EtOH: Ex, excitation; Em, emission. Ex (λ_{max} 389 nm) and Em (λ_{max} 462 nm) were obtained for **4a**.

TABLE III. ^{13}C and ^{1}H Chemical Shifts of **4a**—**f**, **5a** and **5f** in DMSO-d_6



Compd. No.	**4a**		**4b**		**4c**		**4d**		**4e**		**4f**		**5a**		**5f**	
A	N_2		N_2		N_2		N_2		N_2		N_2		NNH_2		NNH_2	
R	MeO		H		Me		Et_2N		AcO		HO		MeO		HO	
C or H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	162.6		159.3		159.5		159.8		159.2		161.5		162.0		160.6	
3	96.7	6.49[s]	99.6	6.56[s]	98.8	6.52[s]	93.9	6.31[s]	98.9	6.52[s]	96.1	6.33[s]	105.8	6.24[s]	102.2	6.18[s]
4	147.0		146.8		146.9		146.6		146.6		147.3		146.8		146.8	
5	124.9	7.65[d]	124.0[a)]		125.1[b)]	7.62[d]	124.2	7.42[d]	124.8	7.78[d]	125.1	7.55[d]	127.4	8.38[d]	127.2	8.29[d]
6	100.9	6.92[q]	123.7[a)]		123.5[b)]	7.16[d]	96.8	6.63[q]	110.4	7.12[q]	108.1	6.77[q]	100.8	6.94[d]	105.0	6.78[d]
7	154.6		132.0		146.9		150.5		153.2		154.7		155.5		155.4	
8	118.8	6.96[d]	116.9		116.9	7.20[s]	108.1	6.46[d]	118.1	7.22[d]	112.2	6.73[d]	111.8	6.97[s]	112.5	6.73[d]
9	159.6		152.7		152.8		155.1		153.4		159.8		160.7		160.4	
10	109.4		115.7		113.4		104.3		113.6		108.1		110.3		109.2	
11	45.9	5.72[s]	45.9	5.90[s]	45.8	5.83[s]	44.7	5.44[s]	46.1	5.86[s]	45.7	5.65[s]	130.9	7.84[s]	131.0	7.82[s]
CH_3O	55.9	3.86[s]											55.7	3.41[s]		
CH_3					21.0	2.40[s]										
$\underline{CH_3}CH_2N$							12.0	1.12[t]								
$CH_3\underline{CH_2}N$							43.7	3.39[q]								
$\underline{CH_3}CO$									20.9	2.31[s]						
$CH_3\underline{C}O$									168.7							
OH																10.47[br]
NH_2														8.14[s]		8.06[s]

Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; br, broad.
a, *b*) Assignments may be reversed in each column.

crystals did not decompose at all after standing for as long as a year at room temperature in a desiccator. No significant change was observed on storage of their 0.5% solutions in chloroform, benzene, or ethanol for at least four days at room temperature.

Spectral Properties

All of the obtained spectra of **4a**—**f** can be well interpreted in terms of the assigned structures, namely, combination of the coumarin nucleus plus diazomethyl functionality. MS of **4** at 70 eV showed a distinct molecular ion peak in every case; most such peaks were abundant, or even the strongest in intensity as compared to fragmentation peaks (see Table I). The infrared (IR) spectra (Table II) exhibited a strong band in the region of 2078—2102 cm^{-1} (KBr tablet) corresponding to the well-known stretching vibration of the diazo N–N bond.

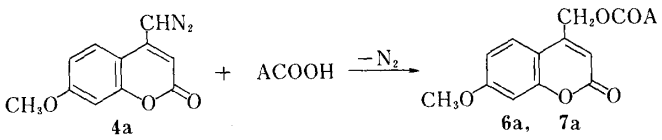
In the UV spectra (Table II), two main absorption bands appeared at 241—254 nm and around 320 nm in ethanol. Significant decreases in these absorptions occurred when very dilute solutions were allowed to stand without protection from light. From the decrease of λ_{max} at 320.5 nm in ethanol and at 319.5 nm in benzene the half-lives of **4b**, for instance, were calculated to be *ca.* 2.5 and 2.0 hours, respectively, at 4×10^{-5} M at 15 °C. No decrease in UV absorption was observed in the dark, or in the case of other related coumarin derivatives without the diazomethyl substituent, suggesting the specific photochemical degradation of **4** in dilute solutions. However, this was not further investigated. Very weak fluorescence owing to the coumarin nucleus can be observed for **4** in ethanol (Table II), with the exception of 7-unsubstituted **4b** which showed no fluorescence.

Proton and carbon-13 nuclear magnetic resonance (NMR) spectral data for **4** are tabulated in Table III. The ^{13}C chemical shifts were assigned with the aid of the off-resonance technique and available data on coumarinoids in the literature.[17] Diazomethyl proton and carbon signals had characteristic values in the regions of δ 5.44—5.90 ppm and δ 44.7—45.9 ppm, respectively, in dimethyl sulfoxide-d_6. Diazomethyl carbons of **4a** and **4f** are shielded by 85.0 and 86.3 ppm, respectively, with respect to the hydrazone carbons of the corresponding coumarin-4-carbaldehyde hydrazones, **5a** and **5f**, measured under the same conditions (see Table III). These data provide additional examples of the extraordinary large upfield shift of the diazomethyl carbon when compared with normal sp^2 hybridized carbon, as reported previously.[18]

Reactivity toward Acid

Examination of chemical behavior has revealed[1] that these compounds (**4**) have sufficient reactivity as diazoalkanes to esterify relatively strongly acidic substances such as carboxylic and sulfonic acids. Takadate *et al.* have also described[13] the reaction of **4a** with carboxylic acids in acetonitrile, giving moderate yields of products. We have examined the reactivity of **4** toward acetic acid in detail by the use of **4a** as a representative, as shown in Table IV. Yields of the acetate ester **6a** were determined by careful titration of the unreacted acid. Although complete conversion of **4a** into **6a** was noted in the reaction with excess acetic acid, only 38% conversion was achieved by the use of excess **4a** (two molar equivalents) at reflux for two hours in chloroform, the most suitable solvent examined. The poor yield was not improved by the use of a larger amount of **4a** or a longer reaction period. However, addition of silica gel catalyst was found to raise the yield to 58%. Almost quantitative yield of **6a** was obtained by refluxing for five hours with a larger amount of silica gel and two molar equivalents of **4a**. Similar features of esterification were noted in the reaction with palmitic acid, in which a 10% yield of the ester **7a** was increased to 86%. In the silica gel-catalyzed esterifications in Table IV, no formation of side product was observed. A control experiment showed that **4a** was negligibly changed in refluxing chloroform in the presence of sillica gel. Thus, the esterification reaction seems to be best carried out in refluxing chloroform with

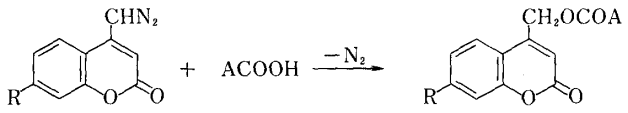
TABLE IV. Reaction[a] of 4-Diazomethyl-7-methoxycoumarin (**4a**) with Carboxylic Acids



Acid A	Solvent	Molar ratio (**4a**/acid)	SiO_2 added (mg)	Reaction time (h)	Yield[b] (%)
CH_3	Dioxane	2	—	2	10
CH_3	Acetonitrile	2	—	2	23[c]
CH_3	Ethanol	2	—	2	23
CH_3	Benzene	2	—	2	37
CH_3	Chloroform	2	—	2	38
CH_3	Chloroform	1/3	—	2	99
CH_3	Chloroform	2	40	2	58
CH_3	Chloroform	2	40	5	98
CH_3	Chloroform	2	20	5	77
CH_3	Chloroform	2	10	5	67
CH_3	Chloroform	1.5	40	5	86
CH_3	Chloroform	1	40	5	61
n-$C_{15}H_{31}$	Chloroform	2	—	2	10
n-$C_{15}H_{31}$	Chloroform	2	40	2	67
n-$C_{15}H_{31}$	Chloroform	2	40	5	86

a) **4a**, 0.2 mmol; solvent, 2 ml.
b) Determined by titration of the unreacted acid with 0.01 N NaOH.
c) Lit.[12] yield, 24.9%.

TABLE V. Esterification Reaction[a] with 4-Diazomethylcoumarins (**4a—f**)



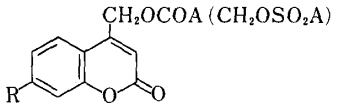
Compd. No.	R	Acid A	Molar ratio (**4**/acid)	Reaction time (h)	Product No.	Yield[b] (%)
4a	MeO	CH_3	2	5	**6a**	95[c]
4a	MeO	n-$C_{15}H_{31}$	2	5	**7a**	84[c]
4a	MeO	C_6H_5	2	5	**8a**	95[c]
4b	H	CH_3	2	5	**6b**	94
4c	Me	CH_3	2	5	**6c**	94
4d	Et_2N	CH_3	1	1	**6d**	97[d]
4d	Et_2N	n-$C_{15}H_{31}$	1	3	**7d**	83
4d	Et_2N	C_6H_5	1	1	**8d**	98[d]
4e	AcO	CH_3	2	5	**6e**	92
4e	AcO	n-$C_{15}H_{31}$	2	5	**7e**	78
4e	AcO	C_6H_5	2	5	**8e**	92
4f	HO	CH_3	2	5	**6f**	84[e]

a) In refluxing chloroform with SiO_2 catalyst.
b) Based on the product isolated.
c) Data reported in the previous communication (ref. 1).
d) Yield in the reaction without SiO_2.
e) Yield in the reaction in refluxing iso-PrOH.

addition of silica gel, which can be removed simply by filtration after the reaction. Table V shows the comparative results of esterification of acetic, palmitic and benzoic acids with **4a**—**f** under the above conditions with the exception of **4f**, which was reacted in refluxing isopropanol because of its poor solubility in chloroform or benzene. As shown in Table V, excellent isolated yields can be achieved, with relatively little difference among the reactions using various reagents, **4a**—**f**. It was recognized, however, that the reaction with **4d** proceeded particularly smoothly with an equimolar amount of the reagent at a shorter reaction period without catalyst. Facile esterification of sulfonic acids using **4a** has already been reported by us.[1)]

Most of the obtained ester products are appreciably fluorescent, as shown in Table VI, which lists their UV and fluorescence spectral data together with their fluorescence quantum yields based on quinine sulfate in ethanol.

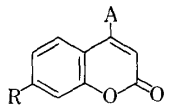
TABLE VI. IR, UV and Fluorescence Spectral Data for Coumarin-4-ylmethyl Esters



Compd. No.	R	A	IR ν_{max}^{KBr} cm^{-1} Ester	UV λ_{max}^{EtOH} nm (log ε)	F[a)] Ex λ_{max} nm	F[a)] Em λ_{max} nm	Quantum yield
6a[b)]	MeO	CH_3	1753, 1721 1243	321 (4.14)	326	396	0.08
7a[b)]	MeO	$C_{15}H_{31}$-*n*	1725, 1170	321.5 (4.18)	325	396	0.10
[b)]	MeO	CH=CH– CH=CHCH$_3$	1715, 1247 1137	262 (4.47) 321.5 (4.16)	324	396	0.12
8a[b)]	MeO	C_6H_5	1718, 1282	321.5 (4.10)	324	396	0.12
[b,c)]	MeO	CH_3	1712, 1350 1178	322.5 (4.11)	325	395	0.04
[b,c)]	MeO	$C_6H_4CH_3$-*p*	1740, 1344 1163	323 (4.16)	323	386	0.01
6b	H	CH_3	1721, 1245	271 (4.02) 311.5 (3.78)	371	445	0.02
6c	Me	CH_3	1724, 1259	279.5 (4.08) 314 (3.98)	378	451	0.05
6d	Et_2N	CH_3	1751, 1708 1241	246 (4.08) 378 (4.30)	379	478	0.48
7d	Et_2N	$C_{15}H_{31}$-*n*	1747, 1708 1140	247 (4.15) 378.5 (4.36)	379	478	0.29
8d	Et_2N	C_6H_5	1712, 1276 1122	236.5 (4.30) 379 (4.33)	379	480	0.32
6e	AcO	CH_3	1728, 1227	276.5 (4.07) 311.5 (4.03)	342	405	0.25
7e	AcO	$C_{15}H_{31}$-*n*	1731, 1243	277 (3.99) 312 (3.94)	331	403	0.14
8e	AcO	C_6H_5	1741, 1722 1269, 1205	275 (4.01) 312 (3.94)	328	403	0.25
6f	HO	CH_3	1745, 1684 1236	325 (4.16)	326	403	0.92

a) Fluorescence spectrum in EtOH: Ex, excitation; Em, emission.
b) Compound reported in the previous communication. IR and UV data were taken from ref. 1.
c) Sulfonate ester.

TABLE VII. Physicochemical and Analytical Data



Compd. No.	A	R	Appearance (Recrystn. solvent)	mp (°C)	Formula	Analysis (%) Calcd (Found)		
						C	H	N
2b	CHO	H	Pale yellow prisms (THF)	156—157 (lit.[a] 155—157)				
2c	CHO	Me	Yellow needles (THF)	200—202	$C_{11}H_8O_3$	70.21 (70.26)	4.29 (4.36)	
2d	CHO	Et_2N	Red prisms (iso-PrOH)	84—85	$C_{14}H_{15}NO_3$	68.55 (69.02)	6.16 (6.31)	5.71 (5.82)
2e	CHO	AcO	Yellow prisms (THF)	188—189	$C_{12}H_8O_5$	62.07 (61.80)	3.47 (3.52)	
3b	CH=NNHTs	H	Colorless prisms (iso-PrOH)	149—153 (dec.)	$C_{17}H_{14}N_2O_4S$	59.64 (59.58)	4.12 (4.01)	8.18 (8.31)
3c	CH=NNHTs	Me	Colorless prisms (iso-PrOH)	147—149 (dec.)	$C_{18}H_{16}N_2O_4S$	60.66 (60.28)	4.53 (4.79)	7.86 (7.71)
3d	CH=NNHTs	Et_2N	Orange prisms (THF)	151—153 (dec.)	$C_{21}H_{23}N_3O_4S$	61.00 (60.85)	5.61 (5.70)	10.16 (9.97)
3e	CH=NNHTs	AcO	Pale yellow prisms (THF)	156—158 (dec.)	$C_{19}H_{16}N_2O_6S$	56.99 (57.00)	4.03 (4.03)	7.00 (6.82)
6b	CH_2OCOCH_3	H	Colorless prisms (iso-PrOH)	102—103	$C_{12}H_{10}O_4$	66.05 (66.16)	4.62 (4.64)	
6c	CH_2OCOCH_3	Me	Pale orange prisms (iso-PrOH)	137—139	$C_{13}H_{12}O_4$	67.23 (67.50)	5.21 (5.23)	
6d	CH_2OCOCH_3	Et_2N	Brown leaves (iso-PrOH)	111—113	$C_{16}H_{19}NO_4$	66.42 (66.58)	6.62 (6.76)	4.84 (4.89)
6e	CH_2OCOCH_3	AcO	Pale yellow prisms (iso-PrOH)	160—162	$C_{14}H_{12}O_6$	60.87 (60.82)	4.38 (4.41)	
6f	CH_2OCOCH_3	HO	Yellow prisms (iso-PrOH)	170—172	$C_{12}H_{10}O_5$	61.54 (61.40)	4.30 (4.37)	
7d	$CH_2OCOC_{15}H_{31}$	Et_2N	Pale brown prisms (iso-PrOH)	73—74	$C_{30}H_{47}NO_4$	74.18 (74.10)	9.75 (9.88)	2.88 (2.84)
7e	$CH_2OCOC_{15}H_{31}$	AcO	Pale yellow prisms (iso-PrOH)	95—97	$C_{28}H_{40}O_6$	71.16 (71.24)	8.53 (8.22)	
8d	$CH_2OCOC_6H_5$	Et_2N	Brown prisms (MeOH)	157—159	$C_{21}H_{21}NO_4$	71.78 (71.85)	6.02 (6.16)	3.99 (4.10)
8e	$CH_2OCOC_6H_5$	AcO	Colorless prisms (AcOEt)	197—199	$C_{19}H_{14}O_6$	67.45 (67.49)	4.17 (4.13)	

a) M. von Strandtmann, D. Connor and J. Shavel, Jr., *J. Heterocycl. Chem.*, **9**, 175 (1972).

A consideration of the comparative merits of the original diazo compounds **4a—f** suggests that **4d** or **4f** is advantageous, leading to highly fluorescent esters. However, the poor solubility of **4f** and relatively low total yield in the synthesis of **4d** are serious disadvantages. In addition, **4d** and **4f** themselves are somewhat fluorescent (quantum yields in ethanol: 0.047 for **4d**; 0.006 for **4f**), while the other diazo compounds have almost no fluorescence (quantum yields <0.001). 4-Diazomethylcoumarin **4b** bearing no 7-substituent does not give rise to fluorescent esters. Thus, among the diazo compounds obtained, **4a** or **4e** bearing a 7-methoxy or 7-acetyloxy substituent, respectively, may be preferable as a practical reagent for fluorescent derivatization. These compounds were shown to be inert to alcohols and phenols

under the present conditions using silica gel, while **4a** was reported[13] to alkylate alcohols in the presence of fluoroboric acid catalyst. Therefore, in view of their extreme stability, reactivity and fluorogenicity, diazo compounds of the present type appear to be practically useful as analytical reagents for carboxylic or sulfonic acids and for alcohols with a suitable catalyst.

Experimental

All melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. IR spectra were determined in KBr tablets using a Hitachi 215 grating spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded at 100 MHz in dimethyl sulfoxide-d_6 on a JEOL JNM-FX-100 spectrometer; chemical shifts are expressed in ppm (δ) downfield from tetramethylsilane as an internal standard. MS were taken at 70 eV on a Shimadzu LKB-900B spectrometer. UV spectra were obtained in EtOH with a Hitachi 200-10 spectrophotometer. In order to obtain accurate UV data for **4a**—**f** great care was taken to protect solutions from light, and to determine spectra as rapidly as possible. Fluorescence spectra were measured in non-fluorescent EtOH on a Shimadzu RF-503 difference spectrofluorophotometer. Relative fluorescence quantum yields were calculated from the UV and fluorescence spectra according to the reported method,[19] based on quinine sulfate in 0.1 N H_2SO_4 (quantum yield = 0.55).

Starting 4-Methylcoumarins(1b—f)——4-Methylcoumarin (**1b**)[20] and 4,7-dimethylcoumarin (**1c**)[21] were prepared according to the literature. 7-Acetyloxy-4-methylcoumarin (**1e**)[22] was obtained from **1f** by acetylation with Ac_2O. Commercially available 7-diethylamino-4-methylcoumarin (**1d**) and 7-hydroxy-4-methyl-coumarin (**1f**) were used.

Coumarin-4-carbaldehydes (2b—e)——General Procedure: Powdered SeO_2 (3.3 g, 30 mmol) was added to a solution of **1b**—**e** (20 mmol) dissolved in a suitable amount of hot dry xylene (30 ml for **2b**, 80 ml for **2c**, 120 ml for **2d**, 200 ml for **2e**) and the whole was refluxed for 8 h with vigorous stirring. The reaction mixture was filtered hot to remove black Se, and the deep orange filtrate was allowed to stand overnight. Almost pure crystals of **2** separated from the solution. Concentration of the filtrate gave a further small amount of crude **2**, which required recrystallization. In the run with **1d**, however, no crystals separated and no solid residue was obtained; therefore, the dark brown residual oil was chromatographed over silica gel using mixtures of hexane and benzene for gradient elution. From the fraction eluted with benzene–hexane (2 : 1 v/v), pure red crystals of **2d** were obtained. Yields: 83% (**2b**), 88% (**2c**), 41% (**2d**), 67% (**2e**). Yields of the products tend to be somewhat lower in larger-scale operations. Melting points and analytical data: see Table VII.

Coumarin-4-carbaldehyde Tosylhydrazones (3b—e)——General Procedure: A mixture of **2b**—**e** (40 mmol) and *p*-toluenesulfonylhydrazine (40 mmol) was suspended in EtOH (100 ml for **3b**—**d**, 250 ml for **3e**) and vigorously stirred at room temperature. After 8 h the precipitates were collected, washed with EtOH, and recrystallized. Yields: 96% (**3b**), 85% (**3c**), 88% (**3d**), 83% (**3e**). Melting points and analytical data: see Table VII.

4-Diazomethylcoumarins (4b—e)——General Procedure: Triethylamine (2 mmol) was added dropwise to a stirred suspension of **3b**—**e** (2 mmol) in MeOH (8 ml) at room temperature, whereupon the initial suspension changed into a solution followed by the gradual appearance of yellow precipitates. The whole was stirred for 2 h (5 h for **4d**), and the resulting precipitates were collected and recrystallized as usual. Yields: 94% (**4b**), 90% (**4c**), 80% (**4d**), 94% (**4e**). Analyses and spectral data: see Tables I—III.

4-Diazomethyl-7-hydroxycoumarin (4f)——Acid-washed and moist activated zinc[23] (4 g) was added to a suspension of **4e** (400 mg) in MeOH (40 ml), and the whole was vigorously stirred for 42 h at room temperature. The zinc was removed by filtration, and washed well with MeOH, then the filtrate and washings were concentrated *in vacuo* to afford **4f** (313 mg, 88%). Recrystallization from THF gave yellow needles. Analysis and spectral data: see Tables I—III.

7-Hydroxycoumarin-4-carbaldehyde Hydrazone (5f)——**2e** (0.23 g, 1 mmol) was added in portions to ice-cooled 80% hydrazine (0.2 g, 3 mmol as NH_2NH_2) diluted with EtOH (5 ml), and the whole suspension was stirred for 1 h at room temperature. The precipitates were collected and recrystallized from EtOH to give yellow prisms (107 mg, 43%), mp 236—239 °C (dec.). *Anal.* Calcd for $C_{10}H_8N_2O_3$: C, 58.82; H, 3.95; N, 13.72. Found: C, 58.98; H, 3.92; N, 13.63. IR ν cm^{-1} (KBr): 3426, 1687, 1280. NMR data: see Table III.

Esterification of Carboxylic Acids with 4-Diazomethylcoumarins (4a—f)——General Procedure: A mixture of 4 mmol of **4**, 2 mmol of acid, and 800 mg of silica gel (Wakogel C-200, 100—200 mesh) in 8 ml of $CHCl_3$ (60 ml of iso-PrOH in the run with **4f**) was vigorously stirred and refluxed for 5 h; the suspension of the reactant gradually changed into a solution. The reaction mixture was filtered to remove the silica gel, washed with $CHCl_3$ and concentrated *in vacuo* to afford the ester product (**6**, **7** or **8**), which was recrystallized. Yields: see Table V. Analyses and spectral data: see Tables VI and VII.

In order to determine the yield of **6a** or **7a** in a small-scale reaction with **4a** (0.2 mmol, see Table IV), the reaction mixture was cooled and diluted with EtOH into 25 ml. A portion of the resulting solution was directly titrated with

0.01 N NaOH using BTB indicator.

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References and Notes

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