Fluorescence Reagents. I.
Derivatization of Carboxylic Acids, Imides and Alcohols with 1-Chloromethylbenz[*c,d*]indol-2(1*H*)-one (CMBI) [1]

Winfried Wendelin*, Gerald Gübitz and Ulrike Pracher

Institut für Pharmazeutische Chemie der Universität Graz,
A-8010 Graz, Austria

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The derivatization and fluorodensitometric determination of carboxylic acids (CA), imides and alcohols with 1-chloromethylbenz[*c,d*]indol-2(1*H*)-one (**19**, CMBI) have been studied. Out of a series of fluorescent fused lactams **9-11**, **13-15** and **17**, benzindolone **17** was selected and transformed *via* hydroxymethylbenzindolone **18** into CMBI **19**. CMBI reacts with CA, diCA and alcohols respectively to yield strongly fluorescent benz[*c,d*]indol-1-ylmethyl esters **20**, **21** (BIM esters) and BIM ethers **22**. Phenobarbital is transformed by action of CMBI into fluorescent 1,3-bisBIM phenobarbital **25**. Studies on the applicability of the derivatization reactions to the fluorodensitometric determination of CA, alcohols and imides showed that CA with more than 3 carbon atoms can be determined *via* BIM esters down to the low picomole range. In the case of alcohols and imides the results were not satisfying. The ir, uv, fluorescence, nmr and mass spectra of the prepared benzindole derivatives are also presented.

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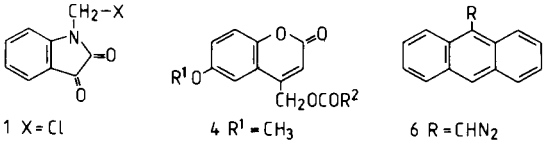
Several years ago *N*-chloromethylisatine (CMI **1**) was found to be an excellent novel reagent for the identification of caboxylic acids (CA) and alcohols, which react with CMI to yield isatinylmethyl esters **2** [2,3] and ethers **3** [4]. The derivatization reaction has also been applied to the quantitative tlc and hplc determination of CA with uv detection [5,6]. Similarly, chloromethylphthalimides, reagents with structural relationship to CMI, have been applied to the labelling of CA [7,8].

The determination method for CA *via* isatinylmethyl esters **2** [5,6] is simple and accurate, but determination procedures with uv detection have relatively high detection limits in comparison with fluorimetric methods [9-15]. For example, the reactions of CA with 4-bromomethyl-7-methoxy- and 7-acetoxycoumarin yield fluorescing esters **4** [9-11] and **5** [12], respectively, action of CA on anthryldiazomethane **6** [13,14] or chloromethylanthracene **7** [15] affords esters **8**, which can be determined by fluorescence measurement down to the low picomole range. Alcohols can be labelled with 1-naphthyl- and 4-(6-methylbenzothiazol-2-yl)phenylisocyanate, respectively [16,17].

Scheme I



1 X = Cl
2 X = OCOR
3 X = OR

4 $R^1 = CH_3$
5 $R^1 = COCH_3$

6 $R = CHN_2$
7 $R = CH_2Cl$
8 $R = CH_2OCOR$

As isatine and its derivatives do not fluoresce, we tried to develop fluorescent reagents of the highly reactive chloromethylamide type. In this paper, the preparation of chloromethylbenzindolone **19** (CMBI) and the reactions of this fluorescence reagent with CA, cyclic imides and alcohols are reported.

Results and Discussion.

Preliminary Experiments.

In order to get on hand fluorescing analogues of isatine and other fused lactams with appropriate properties we prepared in a first series of experiments, according to the literature specified in square brackets, α- and β-naphthisatine **9**, **10** [18,19], 1,8-naphthisatine **11** [20,21], carbostyril **13** [22], phenanthridone **14** [23,24], naphthosultam **15** [25,26] and naphthostyril **17** [27]. The reaction of α-naphthylamine with oxalyl chloride, which should, according to Haller [18,19], afford α-naphthisatine **9**, gave **9** in only 21% yield, the main product was 1,8-naphthisatine

Scheme II

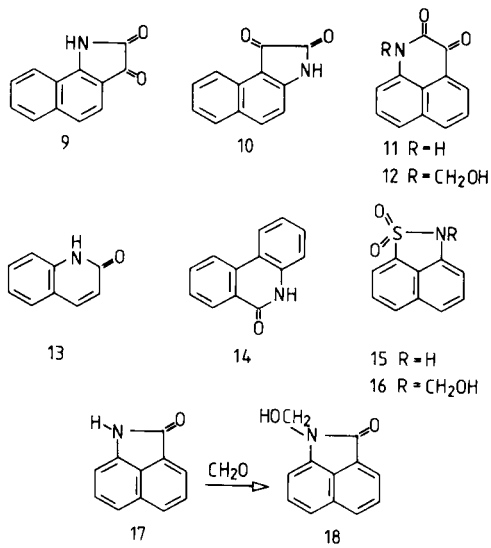


Table I

Maxima of λ_{ex} and λ_{em} and Approximate Detection Limits of the Cyclic Amides **9-11**, **13-15** and **17** (Detection Limits Determined at λ_{em} by Repeated Diluting of the Solutions to the Tenfold Volume Each)

Compound	Maxima of $\lambda_{ex}/\lambda_{em}$ [nm]	Approximate Detection Limits in EtOH (ng/ml)	on Tlc Plates (ng/spot)	Hue of Fluorescence (λ_{ex} = 366 nm)
9	335/395	250	20	orange-pink
10	290/325	2500	250	
11	420/535	5	2	reddish-yellow
13	335/370 (comp [31])	25	>50	bluish
14	320/360 (comp [28,29])	5	5 (comp [29])	light yellow
15	345/445	250	50	greenish
17	380/475 (comp [28])	5	0.5 (measurement with increased sensitivity)	bluish/light yellow

11 (yield 33%). On the other hand, we were not able to prepare pure **11** by reaction of the sodium salt of *N*-(2-naphthyl)-*p*-toluenesulfonamide **20** with oxalylchloride according to Schirmacher and Renn [21].

A preliminary test of the fluorescence properties of the cyclic amides **9-11**, **13-15** and **17**, which were only partially known [28-33], yielded the results given in Table I. Accordingly, 1,8-naphthisatine **11**, phenanthridone **14** (compare [28-30]) and naphthostyril **17** [28] exhibit a strong, α-naphthisatine **9**, carbostyril **13** [31-33] and naphthosultam **15** a weak, and β-naphthisatine **10** a very weak fluorescence in ethanolic solution and on tlc plates after excitation with uv light. The differences between λ_{ex} and λ_{em} (compare Table I) are sufficient for analytical applications in the case of the lactams **11**, **15** and **17**, but not in the case of **9**, **10**, **13** and **14**.

Considering these findings, we selected 1,8-naphthisatine **11**, naphthosultam **15** and naphthostyril **17** as potential starting materials for preparing fluorescent reagents of the chloromethylamide type. However, we were not able to prepare the *N*-hydroxymethyl derivatives **12** and **16** as precursors of the corresponding *N*-chloromethyllactams from 1,8-naphthisatine **11** and naphthosultam **15** according to known methods for the hydroxymethylation of carboxamides [34-37] and sulfonamides [38,39].

Chloromethylbenzindolone **19**, Benzindolylmethyl Esters **20**, **21** and Benzindolylmethyl Ethers **22**.

In contrast, naphthostyril **17** [benz[*c,d*]indol-2-(1*H*)-one], which exhibits the strongest fluorescence of the tested fused lactams, reacts with formaldehyde in dioxane as solvent to afford *N*-hydroxymethylbenzindolone **18** (HMBI) in high yield, if potassium carbonate is employed as catalyst.

1-Chloromethylbenz[*c,d*]indol-2(1*H*)-one (**19**, CMBI) was prepared in almost quantitative yield by reacting HMBI with excess of thionyl chloride or phosphorus pentachloride at low temperatures. Dryly stored, CMBI, a beige substance of melting point 141°, is stable. By action of water and alcohols CMBI is readily transformed into HMBI **18** and ethers **22**, respectively.

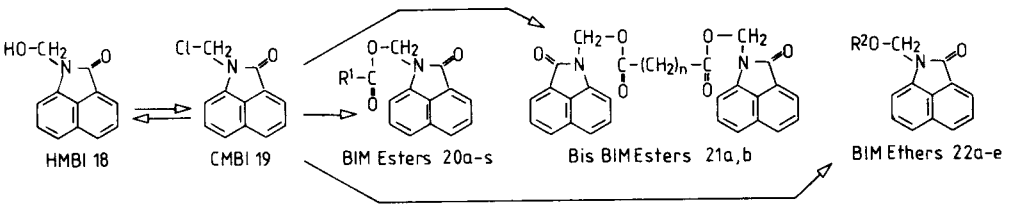
CMBI is a potent reagent for the identification of CA, imides and alcohols. Salts of mono- and diCA react with CMBI in DMF as solvent to afford fluorescent 2-oxo-1,2-dihydrobenz[*c,d*]indol-1-ylmethyl esters **20** and **21**, respectively, for short BIM esters. In half micro preparations a reaction time of 15 minutes at 100° was employed, though, according to tlc, in most of the experiments the reaction was already finished within 10 minutes at room temperature (example: BIM acetate **20b**). Alternatively, one can prepare BIM esters **20** and **21** starting from the acids, if potassium hydrogen carbonate is employed as catalyst (example: BIM stearate **20k**). The BIM esters (and analogously the ethers, see below) are precipitated by addition of water. Excess of CMBI reacts to yield HMBI **18**, which remains dissolved.

Fifteen BIM esters of saturated and unsaturated aliphatic, araliphatic and aromatic mono- and diCA **20a-d,g,i-l,n-p,r,s**, **21b**, (see Table II and III), all of them of yellow low colour, were prepared. In comparison with isatinylmethyl esters **2** [3] they have low melting points. BIM hexanoate **20f** (melting point below 10°) precipitated as oil. Bim valerate, decanoate, oleate and *p*-methoxybenzoate **20e,h,m,q** as well as BIM oxalate **21a** were prepared only on an analytical scale, see Experimental.

Alcohols react with CMBI to afford fluorescing BIM ethers **22**. The reaction can be accomplished by boiling of CMBI in excess of the alcohol concerned (example: BIM methyl ether **22a**) or by heating of CMBI with equimolar

Table II

Melting Points, Yields, Recrystallization Solvents, Rf Values (Tlc), Analytical Data, Ir and Uv Bands of HMBI **18**, CMBI **19**, 2-Oxo-1,2-dihydrobenz[*c,d*]indol-1-ylmethyl Esters (BIM Esters) **20a-d,g,i-l,n-p,r,s**, **21b**, and BIM Ethers **22a,b,e**



Compound	R^1/R^2	Mp °C	Yield %	Recrystall. Solvent	Rf (tlc)	Molecular Formula	Analyses % Calcd./Found [f] C	H	N	IR (Potassium Bromide) cm^{-1}	UV [g] λ max [nm] (ε)
18		141	70	ethanol	0.03 [d]	$C_{12}H_9NO$	72.35 72.41	4.55 4.46	7.03 7.06	3350 (OH), 1670 (lactam), 1630, 1067, 1053 (C-O), 1008, 785	210 (32495), 248 (19670), 273 (5176), 336 (4141)
19		141	90	cyclohexane	0.27 [d]	$C_{12}H_8ClNO$	66.22 66.31	3.70 3.70	6.44 6.29	1720 (lactam), 1635, 1490/1475, 1285, 780	249 (23892), 273 (6729), 335 (5066), 356 (3970)
BIM esters										Ester, lactam, CH-out of plane frequencies, other bands	
20a	H	135	77 [b]	ethanol water 5:1	0.35 [d]	$C_{13}H_9NO_3$	68.72 68.62	3.99 3.99	6.16 6.10	1725, 1715, 770/760, 1490/1470, 1180/1160, 1125	252 (24580), 272 (5830), 336 (4920), 360 (3820)
20b	CH_3	125	60 [b]	ethanol water 5:1	0.38 [d]	$C_{14}H_{11}NO_3$	69.70 69.42	4.60 4.60	5.81 5.90	1745, 1715, 770, 1635, 1493/1470, 1220, 960, 825	249 (27165), 272 (7412), 336 (5666), 356 (4502)
20c	C_2H_5	53	30 [b]	ethanol	0.41 [d]	$C_{15}H_{13}NO_3$	70.58 70.28	5.13 4.90	5.49 5.65	1740, 1715, 770, 1630, 1490/1470, 1160/1148, 992	250 (27898), 274 (7831), 336 (5873), 359 (4650)
20d [a]	n-C_3H_7	63	56 [b]	methanol	0.44 [d]	$C_{16}H_{15}NO_3$	71.36 71.59	5.62 5.42	5.65 5.18	1740, 1715, 780/770, 1500, 1475, 1160, 960, 830	246 (26000), 336 (5500), 353 (4500)
20g [a]	n-C_7H_{15}	40	30 [b]	ethanol	0.50 [d]	$C_{20}H_{23}NO_3$	73.82 74.10	7.12 7.11	4.30 4.41	1740/1725, 775, 2925 (CH), 1497/1473, 1145, 828	
20i	n-$C_{11}H_{23}$	55	30 [b]	triturated with methanol	0.53 [d]	$C_{24}H_{31}NO_3$	75.56 75.30	8.19 7.94	3.67 3.79	1740/1725, 775, 2920, 2850 (CH), 1498/1475, 828	
20j	n-$C_{15}H_{31}$	64	74 [b]	ethanol	0.55 [d]	$C_{28}H_{39}NO_3$	76.85 76.66	8.98 9.01	3.20 3.19	1730/1710, 768, 2908, 2850 (CH), 1495/1470, 822	

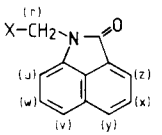
Table II (continued)

Compound	R^1/R^2	Mp °C	Yield %	Recrystall. Solvent	Rf (tlc)	Molecular Formula	Analyses % Calcd./Found [f] C	H	N	IR (Potassium Bromide) cm^{-1}	UV [g] λ max [nm] (ε)
20k	n-$C_{17}H_{35}$	58	88	ethanol	0.56 [d]	$C_{30}H_{43}NO_3$	77.37 77.81	9.31 9.28	3.02 3.10	1740/1730, 775, 2930, 2860 (CH), 1640, 1475, 830	250 (24870), 272 (7047), 321 (4050), 336 (5265)
20l [a]	$CH_2=CH$-	80	68 [b]	methanol-water 3:1	0.40 [d]	$C_{15}H_{11}NO_3$	71.12 71.12	4.38 4.19	5.55 5.53	1740, 1715, 770, 1632, 1495/1470, 1165, 1155 820	209 (42720), 249 (24270), 272 (6490), 336 (4850)
20n	C_6H_5	166	68 [b]	ethanol	0.45 [d]	$C_{19}H_{13}NO_3$	75.24 75.32	4.32 4.38	4.62 4.68	1715, 785, 770, 715, 1245, 1090/1070, 930/920	213 (44000), 244 (29160), 272 (8850), 336 (6510)
20o	p-ClC_6H_4	191	78 [b]	ethanol	0.51 [d]	$C_{19}H_{12}ClNO_3$	67.56 67.25	3.58 3.50	4.15 4.15	1725, 1705, 850, 760, 1495, 1250, 1080, 1010	248 (39040), 320 (3430), 336 (4790), 378 (2740)
20p [a]	p-$CH_3C_6H_4$	193	68 [b]	ethanol	0.45 [d]	$C_{20}H_{15}NO_3$	75.69 75.11	4.76 4.73	4.41 4.67	1720, 1705, 775, 755, 1495, 1250, 1175, 1015	248 (34900), 320 (3190), 336 (4490), 378 (2960)
20r	p-$NO_2C_6H_4$	221	70 [b]	benzene	0.47 [d]	$C_{19}H_{12}N_2O_5$	65.52 65.69	3.47 3.27	8.04 7.81	725, 1705, 780, 720, 1535 (NO_2), 1500, 1260 910	248 (30360), 318 (3850), 333 (4620)
20s [a]	$C_6H_5CH_2$	82	60 [b]	ethanol-water	0.45 [d]	$C_{20}H_{15}NO_3$	75.70 76.36	4.76 4.91	4.41 4.42	1740, 1710, 775, 705, 1495/1470, 1305, 1125 960	252 (30130), 320 (3850), 336 (5130)
21b	n = 2	158	80 [b]	triturated with $KHCO_3/H_2O$	0.38 [d]	$C_{28}H_{20}N_2O_6$	69.99 69.93	4.20 4.05	5.83 5.97	1745/1735, 1715/1705, 825, 775, 1495, 1145, 955	253 (51720), 320, (8620), 336 (10920)
BIM ethers										Lactam, C-O, CH-out of plane fr, other bands	
22a	CH_3	107	82 [c]	methanol	0.35 [e]	$C_{13}H_{11}NO_2$	73.22 73.07	5.20 5.20	6.57 6.53	1715, 1082, 770, 1635 1495/1470, 1390, 1005	250 (23062), 272 (6187), 335 (4687), 357 (3712)
22b [a]	C_2H_5	69	50 [c]	ethanol	0.57 [e]	$C_{14}H_{13}NO_2$	73.90 73.91	5.76 6.02	6.16 5.91	1705, 1075, 765, 1635, 1495/1475, 1380, 1290 1005	254 (23180), 274 (5660), 336 (4420), 357 (3480)
22e [a]	n-$C_{18}H_{37}$	66	77 [c]	ethanol	0.74 [e]	$C_{30}H_{46}NO_2$	79.77 79.86	10.04 10.30	3.10 3.15	1710, 1095, 775, 2920 2860 (CH), 1640, 1475 1300	254 (29650), 275 (5660), 338 (6010), 360 (4810)

[a] For preparation and Rf values of **20e** (R^1 = n-C_4H_9), **20f** (R^1 = n-C_5H_{11}), **20h** (R^1 = n-C_9H_{19}), **20m** (R^1 = 9-Heptadecenyl), **20q** (R^1 = 4-$CH_3OC_6H_4$), **20t** (R^1 = HOOC), **20u** (R^1 = $HOOC(CH_2)_2$), **21a** (n = 0), **22c** (R^2 = n-C_4H_9), **22d** (R^2 = n-C_6H_{13}), **22f** (R^2 = *sec*-Butyl) and **22g** (R^2 = *t*-Butyl) see Experimental and (nmr and ms of **20f**) Table III. [b,c] Preparation according to the procedure described for [b] **20b**, [c] **22a**. [d,e] Elution solvents: [d] benzene-ether 90:20; [e] benzene-methanol-ether 90:5:10. [f] Calculated and found values of chlorine of **19** and **20o** also agreed within small margins (< 0.4%). [g] Solvent in case of **18**, **20c**, **20n** and **22a**: ethanol; **22l**: methanol; other compounds: chloroform.

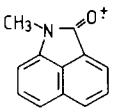
Table III

Proton Chemical Shifts and Mass Spectral Fragments of **17**, HMBI **18**, CMBI **19**, Benzindolylmethyl Esters **20** and **21**, Ethers **22**

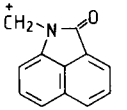


18-22

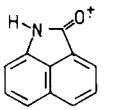
18 X = OH
19 X = Cl
20 X = $OCOR^1$
21 X = BIM$OCO(CH_2)_nCOO$
22 X = OR^2



23^+, m/e 183
(M^+-R^1COO+H
or M^+-R^2O+H)



24, m/e 182
(M^+-R^1COO
or M^+-R^2O)



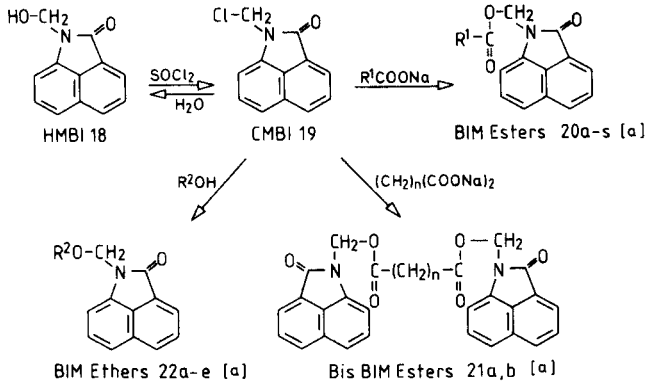
17^+, m/e 169
(M^+-R^1COOCH
or M^+-R^2OCH)

Compound	Proton Bands, ppm [a,b]								Mass Spectral Fragments: m/e (Relative Intensities)					
	u [c]	v [c]	w [d]	x [d]	y [c]	z [c]	r [e]	Protons of X (OH, R^1COO, R^2O)	M:	23:	24	17:	Further Fragments	
17	7.12	7.58	7.70	7.87	8.15	8.25	–	(NH 10.0-12.0)						
18	7.38	7.63	7.75	7.89	8.19	8.28	5.43	OH 5.0-8.0						
19	7.10	7.50	7.62	7.70	8.04	8.06	5.82							
20a	7.12	7.47	7.56	7.72	8.12	8.18	6.04	H(OCHO-) 8.16 s	217 (100)	183 (33)	182 (53)	169 (25)	219 (36)	154 (34)
20b	7.37	7.64	7.75	7.86	8.17	8.29	6.00	CH_3 2.60 s	241 (75)	183 (78)	182 (91)	169 (100)	127 (63)	43 (62)
20d	7.16	7.47	7.53	7.68	8.04	8.09	6.00	n-C_3H_7 0.9 t, 1.65 m, 2.31 t (J = 7)	269 (44)	183 (18)	182 (96)	169 (100)	127 (56)	43 (40)
20f	7.32	7.60	7.73	7.84	8.18	8.25	6.00	n-C_5H_{11} 0.82 t, 1.15 b, 1.50 m, 2.33 t	297 (7)	183 (6)	182 (20)	169 (25)	99 (11)	71 (20) [f]
20k	7.20	7.50	7.61	7.73	8.07	8.14	6.00	$nC_{17}H_{35}$ 0.87 t, 1.22 b, 1.64 m, 2.32 t	465 (10)	183 (98)	182 (100)	169 (98)	267 (4)	127 (30) [g]
20n	7.20 – 7.60		(3H)	7.72	8.04	8.13	6.24	C_6H_5 7.2-7.6 (3H), 8.04 (d, 2H, J = 8)	303 (17)	183 (14)	182 (39)	169 (11)	105 (100)	77 (54)
20o	7.32	7.46	7.66	7.78	8.14	8.16	6.24	4-ClC_6H_4 7.40 (d, 2H, J = 8), 7.98 (d, 2H)						
20p	7.3?	7.54	7.64	7.78	8.10	8.18	6.26	p-$CH_3C_6H_4$ 2.36 s, 7.24 d, 7.97 d (J = 9)						
20s	7.08	7.43	7.56	7.70	8.05	8.11	6.00	$CH_2C_6H_5$ 3.62 (s, 3H), 7.24 (s, 5H)	317 (26)	183 (22)	182 (100)	169 (24)	127 (36)	91 (38)
21b	7.13	7.49	7.60	7.72	8.07	8.12	5.97	$CO(CH_2)_2CO$ 2.66 s		183 (45)	182 (98)	169 (100)	127 (78)	28 (98) [h]
22a	7.30	7.63	7.78	7.90	8.20	8.31	5.33	CH_3 3.35 s	213 (55)	183 (98)	182 (100)	169 (27)	212 (M^+-1,98)	154 (86)
22b	7.10	7.50	7.60	7.73	8.08	8.13	5.40	CH_3CH_2 1.18 (t, 3H), 3.62 (qua, 2H, J = 7)	227 (36)	183 (100)	182 (89)	169 (59)	154 (27)	127 (59)
22e	7.10	7.48	7.58	7.70	8.04	8.09	5.38	n-$C_{18}H_{37}$ 0.85 t, 1.24 b, 1.52 m, 3.54 t	451 (9)	183 (22)	182 (24)	169 (97)	199 (**18**:,15)	127 (25) [i]

[a] Nmr solvent for **17**, **18**, **20b**, **c**, **f**, **g**, **i**, **j** and **22b**: DMSO-d_6; other compounds: deuteriochloroform. [b] Nmr of **20c**, **1**: signals for u-z and r approx. as in the case of **20b**; CH_3CH_2 (**20c**) 1.04 t, 2.37 q, CH_2 = CH- (**20l**) 5.7-6.7. **20g**, **i** and **j**: spectra are approx. identical with the one of **20f**, but the intensities of the signals for $(CH_2)_n$ are 8, 16 and 24. [c] Doublets with J = 8Hz. [d] Triplets with J = 8 Hz. [e] Singlets. [f] 87 (78), 73 (98), 60 (100), 43 (98), 41 (98). [g] Ion series $C_nH_{2n+1}^+$: 183 (98)-29 (25); C_nH_{2n}:: 182 (100)-28 (75); $C_nH_{2n-1}^+$: 181 (7)-41 (60). [h] 267 (Parent peak, 15), 227 (81), 199 (55). [i] Ion series $C_nH_{2n+1}^+$: 253 ($C_{18}H_{37}^+$, 9) -43 (98); C_nH_{2n}:: 252 ($C_{18}H_{36}$:, 39) -42 (60); $C_nH_{2n-1}^+$: 97 (100) -41 (91).

amounts of the alcohols in DMF as solvent in the presence of potassium hydrogen carbonate (example: BIM octadecyl ether **22e**). The BIM ethers **22a,b** and **e** are yellow compounds, which melt at relatively low temperatures (Table II and III) in comparison with isatinylmethyl ethers **3** [4]. BIM butyl and BIM hexyl ether **22c,d** are oils at room temperature. BIM *sec*-butyl and BIM *t*-butyl ether **22f,g** were prepared only on an analytical scale.

Scheme III

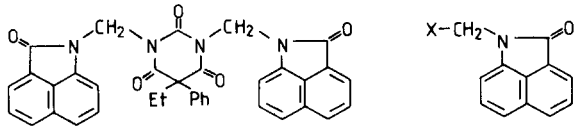


[a] For R^1 (20a-s), R^2 (22a-e) and n (21a,b) see Table II

CMBI **19** also reacts with the salt of imides to yield fluorescent N-BIM imides. Thus, sodium 5-ethyl-5-phenylbarbiturate (phenobarbital Na) is transformed by action of CMBI **19** (1:1) in DMF as solvent into 1,3-bis BIM-5-ethyl-5-phenylbarbituric acid (**25**) in 80% yield.

With tertiary amines, CMBI reacts to afford fluorescing BIM ammonium chlorides **26**. The reaction is applicable to the quantitative determination of drugs with tertiary amino groups, see [40]. Action of ammonium thiocyanate on CMBI yields 1-isothiocyanomethylbenz[*c,d*]-indol-2(1*H*)-one (**27**, IMBI), which is useful as fluorescent reagent for primary and secondary amines. These add to **27** to yield BIM thioureas **28**, see [41].

Scheme IV



25

Et = C_2H_5, Ph = C_6H_5

23 X = H
26 X = $\overset{(+)}{N}R^1R^2R^3Cl^-$
27 X = NCS
28 X = $NHCSNR^1R^2$

IR, UV, Fluorescence, NMR and Mass Spectra of BIM Esters **20** and Ethers **22**.

IR Spectra (Table II).

Bands characteristic of the acyloxymethyl and alkoxymethyl moiety of BIM esters **20** and ethers **22** appear in the regions of 1720-1740 cm^{-1} (C=O) and 1075-1100 cm^{-1} (C-O-C), respectively. Peaks characteristic of the benzindolylmethyl part of **20** and **22** are observed especially in the regions of 3000-3060 (CH, aromatic), 1670-1720 (lactam), 1470-1500 (CH_2-bending vibrations), 1360-1410 and 820-840/760-790 cm^{-1} (CH-out of plane vibrations).

UV Spectra (Table II).

Compounds **18-22** in each case show an intense absorption band at about 250 nm, a shoulder at about 270 nm, and weak absorptions at about 320, 336 and 355 nm. All these bands are characteristic of the benzindolone moiety.

Fluorescence Spectra.

Benzindolones **17-22** show a high flourescence intensity in ethanolic solution and on tlc plates (Table I). The quantum yield of *N*-methylbenzindolone **23**, representing the fluorescing basis component of all compounds, has been stated to be 0.086 [28]. In Figure 1 the fluorescence spectrum of BIM stearate **20k**, recorded on a tlc plate, is presented. The excitation spectrum shows a maximum at 365 nm, a shoulder at 340 nm, and a weak band at 285 nm. The emission maximum appears at 480 nm, a shoulder can be seen at 460 nm. The difference between excitation and emission maximum is large enough for analytical applications.

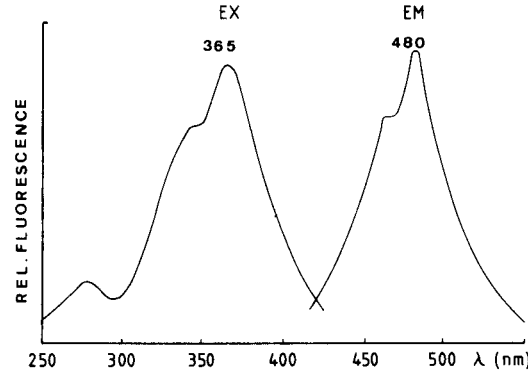


Figure 1. Excitation and emission spectrum of BIM stearate **20k**, recorded on a tlc plate (50ng/spot).

NMR Spectra (Table III).

The signals for the aromatic protons u-z of the benzindole ring system form a characteristic pattern. Protons u and z (at C-8 and C-3) are in all compounds maximum shielded and deshielded, respectively, because of the resonance donating nitrogen atom and resonance withdrawing carbonyl group in the neighbouring positions 1 and 2.

Mass Spectra (Table III).

In the mass spectra of BIM esters **20** peaks of diagnostic value to the identification of the esterified CA appear for molecule ions 20^+ (exception: BIM succinate **21b**) and, in most cases, for acyl cations R^1CO^+ and alkyl cation R^{1+}. In

the mass spectra of BIM ethers **22** the peaks representing molecule ions **22**$^{+\cdot}$ are characteristic of the etherified alcohol. Partly complementary, the fragmentation of BIM esters **20**, **21** and ethers **22** also yields $BIMO^+$ ions (m/e 198), methylbenzindolone ion radicals **23**$^{+\cdot}$, BIM^+ ions **24**, benzindolone ion radicals **17**$^{+\cdot}$ and naphthyl cations (m/e 127), which cause the most intense peaks in the mass spectra.

Studies on the Quantitative Fluorodensitometric Evaluation.

Carboxylic Acids.

As described above, BIM esters **20** and **21** show a high fluorescence intensity on tlc plates. We therefore tested the applicability of this derivatization reaction to the fluorodensitometric determination of CA. When we transformed the reaction to the analytical scale, the application of a high excess of reagent and the use of a catalyst were found to be necessary. As catalysts triethylamine, potassium hydrogen carbonate and combinations of potassium hydrogen carbonate and crown ethers were tested. The best results were obtained using acetone as solvent and potassium hydrogen carbonate/dibenzo-18-crown-6 as catalyst and employing the conditions, which are given in the Experimental under "Derivatization Procedure for the Fluorodensitometric Evaluation of Carboxylic Acids on Analytical Scale". Figure 2 shows the different rates of formation of BIM stearate in the reaction of stearic acid with CMBI without and with potassium hydrogen carbonate/dibenzo-18-crown-6 catalysis. Using a twentyfold excess of the reagent, a reaction time of 15 minutes at a temperature of 50° was found to be sufficient in most of the experiments.

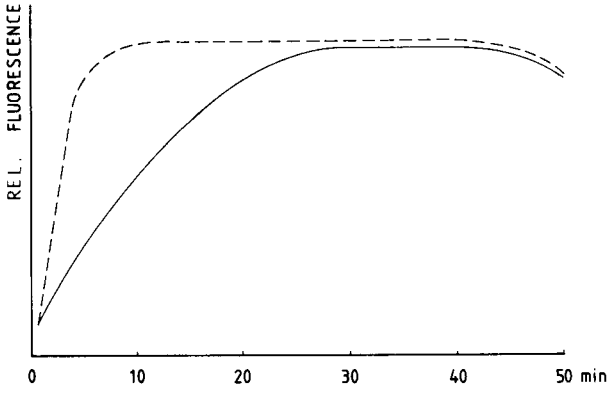


Figure 2. Kinetics of the reaction of the stearic acid with CMBI (twentyfold excess) in acetone at 50°. Without catalyst_____. With dibenzo-18-crown-6/potassium hydrogen carbonate as catalyst_____.

In order to determine the yields under analytical conditions, the amounts of BIM esters formed in the reaction mixtures were determined by means of tlc with fluorodensitometric evaluation using solutions of the pure derivatives as standards. In case of benzoic acid, for example, a 96 percent conversion into BIM benzoate **20n** could be observed. Figure 3 shows a tlc of some CA after reaction with CMBI. As can be seen from the chromatogram, impurities of the reagent and/or by-products formed during the reaction and/or the development of the chromatogram interfere with the quantitative determination of the lower CA up to 3 carbon atoms. Possibly these shortcomings can be overcome by use of a reagent of highest purity and/or by further variation of the reaction conditions.

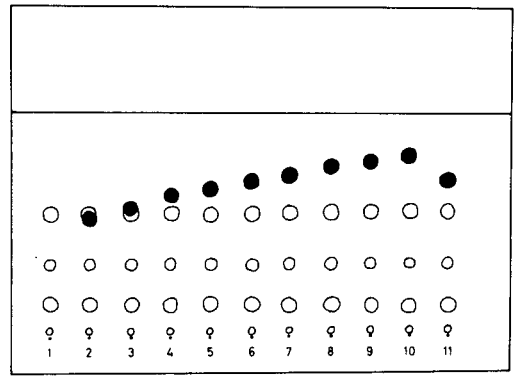


Figure 3. Tlc of the reaction mixtures of ten fatty acids with CMBI. Elution solvent: cyclohexane-chloroform-diethyl ether (20:20:80). 1, Blank; 2, formic acid; 3, acetic acid; 4, propionic acid; 5, butyric acid; 6, hexanoic acid; 7, lauric acid; 8, myristic acid; 9, palmitic acid; 10, stearic acid; 11, benzoic acid.

A scan of a chromatogram of stearic acid after transformation into BIM stearate **20k** is shown in Figure 4.

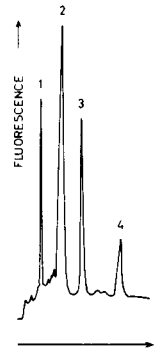


Figure 4. Scan of a chromatogram of stearic acid after reaction with CMBI. 1,2,3: By-products; 4: BIM stearate [1ng (2.15 pmol)/spot].

The detection limits, obtained for higher fatty acids, are about 1 pmol/spot or 25 pmol of fatty acid/50 μl reaction mixture, respectively. Linearity is observed over a range of one and a half decades. The correlation coefficients lie between 0.996 and 0.999. A calibration curve for stearic acid for the concentration range between 0.5 and 10 ng/spot is shown in Figure 5. The reproducibility, which is

expressed by the relative standard deviation, was, for stearic acid, found to be 2.6% for 50 ng/spot and 5.8% for 5 ng/spot (n=6).

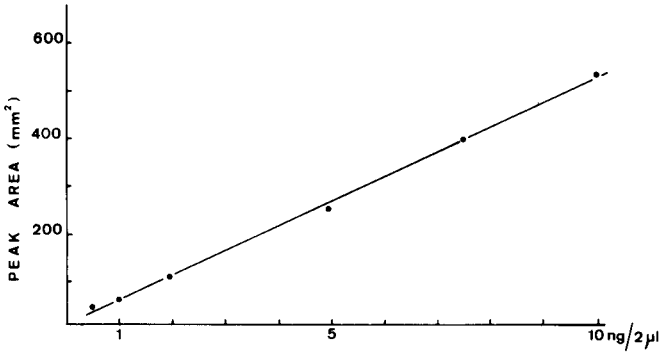


Figure 5. Calibration curve for stearic acid after derivatization with CMBI in the range from 0.5 to 10 ng/spot.

As preliminary experiments have shown, the described derivatization method could also be applicable to the determination of CA *via* BIM esters **20**, **21** if hplc for separation and fluorescence detection is used.

Imides and Alcohols.

As described above, imides, in analogy to CA, react with CMBI to give highly fluorescent derivatives. Problems however, occur, when the quantitative evaluation of the developed spots on the tlc plates is attempted. In particular, the BIM derivatives of barbiturates, for example bis BIM phenobarbital **25**, are more polar than BIM esters **20** and cannot be separated from the polar by-products, which are formed by decomposition of excess reagent on the tlc plates. Therefore, a quantitative analysis of barbiturate with the elution solvents applied is not possible. Similarly, the reaction of CMBI with alcohols yielding fluorescent BIM ethers **22** is suitable for the qualitative analysis and characterization of alcohols, but so far it was not possible to adapt it to the quantitative determination. Due to the fact that under analytical conditions, with highly diluted solutions and excess of reagent, the yields of BIM ethers are below 50%, a sufficient reproducibility for quantitative determinations is not ensured.

EXPERIMENTAL [42]

Melting points were determined on a Kofler melting point apparatus. Thin-layer chromatograms were run on Polygram SIL G/UV 254-plates (Macherey-Nagel and Co). Elution solvent I (e.s. I): benzene-ether 90:20; e.s. II: benzene-methanol-ether 90:5:10. The developed spots were detected by visual examination under uv light. Ultraviolet spectra (λ max [nm], ε-values in round brackets) were obtained using a UV/VIS spectrophotometer Perkin-Elmer model 320. Infrared spectra were recorded with a Perkin-Elmer 225 grating-spectrophotometer. Nuclear magnetic resonance spectra were taken on a Perkin-Elmer R 32 instrument. Chemical shifts are reported as δ-units (ppm) with sodium (3-trimethylsilypropanoate-d_4) or tetramethylsilane as internal standard. Mass spectra were run on a Varian-311A spectrometer (EI, 70 eV, R 1000). "BIM" is the abbreviation for 2-oxo-1,2-dihydrobenz[*c,d*]indol-1-ylmethyl. Elemental analyes were performed by Institute of Organic Chemistry, Graz, Austria.

1*H*-Benz[*g*]indole-2,3-dione (**9**, α-Naphthisatine) and 1*H*-Benzo[*d,e*]-quinoline-2,3-dione (**11**, 1,8-Naphthisatine) (Compare Preparation of **9** [18,19]).

In a three-necked 200 ml flask equipped with reflux condenser, stirrer and thermometer, 14 g (0.06 mole) of *N*-(α-naphthyl)oxamoyl chloride (preparation see [18,19]) are dissolved in 65 g of nitrobenzene. Aluminum chloride (14 g, 0.105 mole) is added under stirring. The mixture is heated and kept at 80° for 5 hours (evolution of hydrochloric acid) and then poured on ice. The nitrobenzene is removed by steam destillation. Then the residue is dissolved in hot 2*N* aqueous sodium hydroxide and filtered. Addition of aqueous hydrochloric acid to *p*H 4 yields a brown precipitate. The latter is treated with 200 ml of hot dioxan whereby 3.9 g (33%) of pure 1,8-naphthisatine **11** remain undissolved. The filtrate is evaporated to dryness and the residue is treated under stirring with an excess of sodium hydrogen sulfite solution. The resulting precipitate is filtered, washed with acetone and decomposed by stirring with diluted hydrochloric acid for 0.5 hours. According to tlc the precipitate formed contains **9** and **11**. Twice repeated recrystallisation from glacial acetic acid yields 2.5 g (21%) of α-naphthisatine **9**.

α-Naphthisatine **9**.

This compound had mp 255° (mp [43] 255°); ir (potassium bromide): 3200 (NH), 1740 (CO), 1725 (amide I), 1630, 1532, 1462, 830, 790, 740 (CH-out of plane vibr); uv (ethanol): 224 (32236), 276 (22946), 465 (1679); nmr (DMSO-d_6): δ 6.7-7.2 (NH, 1H), 7.2-7.8 (aromatic, 4H), 7.90 (dd, H-9, 1H, $J_{8,9}$ = 8 Hz, $J_{7,9}$ = 2 Hz), 8.17 (d, H-4, 1H, J = 8 Hz).

1,8-Naphthisatine **11**.

This compound had mp 296-299° (mp [44] 295-300°); uv, ir: see [44]; nmr (DMSO-d_6): δ 7.36 (d, H-9, 1H), 7.56 (d, H-7, 1H), 7.75 (t, H-5, 1H), 7.83 (t, H-8, 1H), 8.41 (d, H-4 and H-6, 2H), J = 8 Hz each; 3.3 (b, NH?, 1H).

3*H*-Benz[*e*]indol-1,2-dione (**10**, β-Naphthisatine).

Compound **10** was prepared according to [18,19], mp 252° (mp [45] 252°); ir (potassium bromide): 3200 (NH), 1750 (CO), 1710 (amide I), 1530, 1230/1208, 830/820/812, 752 (CH-out of plane vibr); uv (ethanol): 214 (34915), 233 (33847), 292 (5339), 358 (6780), 463 (1068); nmr (DMSO-d_6): δ 7.10 (d, H-4, 1H), 7.37 (t, H-7, 1H), 7.62 (t, H-8, 1H), 7.82 (d, H-9, 1H), 8.12 (d, H-6, 1H), 8.34 (d, H-5, 1H), J = 9 Hz each; 10.6 (b, NH, 1H).

2*H*-Naphth[1,8-*c,d*]isothiazol 1,1-Dioxide (**15**, Naphthosultam).

Compound **15** was prepared according to [25,26], mp 178° (mp [26] 177-178°); nmr (DMSO-d_6): δ 3.85 (s, NH, 1H), 6.85 (dd, H-3, 1H, $J_{3,4}$ = 8 Hz, $J_{3,5}$ = 2 Hz), 7.45-7.55 (H-4 and H-5, 2H), 7.77 (t, H-7, 1H), 8.00 (d, H-6, 1H), 8.15 (d, H-8, 1H), $J_{6,7}$ = $J_{7,8}$ = 8 Hz.

Benz[*c,d*]indol-2(1*H*)-one (**17**, Naphthostyril).

Compound **17** was prepared in 80% yield by the method of Harnisch [27], mp 181° (mp [46] 180.4-181.4°); ir (potassium bromide): 3180 (NH), 1710/1690 (amide I), 1640, 1492/1468, 1260, 1080, 828, 776, 680 (CH-out of plane vibr); uv [44]; nmr: Table III.

1-Hydroxymethylbenz[*c,d*]indol-2(1*H*)-one (**18**, HMBI).

Naphthostyril **17** (8 g, 0.047 mole) is dissolved with stirring in 200 ml of dioxane at 60°. If the solution is not clear, it is filtered. Aqueous formaldehyde (35%, 10.2 g, 0.118 mole) and potassium carbonate (16.2 g, 0.118 mole) are added, and the mixture is heated and kept at 80° for 30 minutes. The potassium carbonate is filtered and the filtrate is evaporated to dryness. The resulting residue is recrystallized from about 50 ml of ethanol to yield 6.3 g (67%) of **18**, yellow hexagonal prisms, mp 141°;

analysis, Rf (tlc), ir, uv: Table II; nmr: Table III.

1-Chloromethylbenz[*c,d*]indol-2(1*H*)-one (**19**, CMBI).

Thionyl chloride (32.12 g, 0.27 mole) is placed in a 100 ml three-necked flask equipped with thermometer, calcium chloride tube and gas exhaust channel and cooled to 0°. Then HMBI **18** (5.97 g, 0.03 mole) is added in portions. If the solution becomes gelatinous, a little more thionyl chloride is employed. The solution is stirred at 0° for 30 minutes and then at room temperature for 90 minutes. Evaporation of excess thionyl chloride at the filter pump and then at the oil pump yields 6.0 g (92%) of **19**, beige crystals, mp 141°. Recrystallization from cyclohexane effects no further purification. Dryly stored, CMBI is stable. **19** is soluble in DMF, acetone and chloroform and slightly soluble in benzene and cyclohexane. With water and methanol (see below) **19** quickly reacts to yield HMBI **18** and BIM methyl ether **22a**, respectively. Accordingly, **19** always decomposes during the development on tlc plates to partly afford HMBI **18**. If methanol is contained in the elution solvent, **19** is partly transformed into BIM methyl ether **22a** during the development. Analysis, Rf (tlc), ir, uv: Table II; nmr and ms: Table III.

CMBI **19** was also prepared by suspending HMBI **18** (0.12 g, 0.06 mmole) in 1 ml of ether, adding phosphorus pentachloride (0.125 g, 0.6 mmole) at 0° and stirring the resulting mixture for 1 hour at 20°. Cooling to 0° (1 hour) and filtering yielded 0.1 g (77%) of nearly pure **19**.

BIM Acetate (**20b**). Example for the Preparation of BIM Esters **20** and **21** from CMBI and Sodium Salts of Carboxylic Acids (Table II, III).

CMBI **19** (0.5 g, 2.3 mmoles) and sodium acetate (0.189 g, 2.3 mmoles) are mixed and ground and placed together with 8 ml of DMF in an Erlenmeyer flask with reflux condenser and calcium chloride tube. The mixture is heated with stirring to 100° and kept at this temperature for 20 minutes (in the case of BIM acrylate **20l**: 10 minutes at 20°). After cooling to 20°, 10 ml of water are added drop by drop. The resulting precipitate is filtered and recrystallized from ethanol-water to yield 0.33 g (60%) of **20b**, yellow rods, mp 125°.

The solid BIM esters **20a,c,d,g,i,j,k,l,n,o,p,r,s** and **21b** (structural formulae see Table II) were analogously prepared. In order to obtain crystalline products the precipitation of the low melting esters **20c,d,g,i,j** and **k** from the reaction mixture with water was accomplished under cooling with ice. Nevertheless BIM octanoate **20g** first precipitated as oil. The oil was extracted with cyclohexane, the solvent was removed and the residue was taken up in hot ethanol. Filtering and cooling of the solution yielded crystals of **20g**. BIM laurate **20i** also precipitated as oil first. The oil was separated and treated with methanol to give crystalline **20i**.

All BIM esters **20** and **21** which are solid at room temperature crystallized as yellow rods or needles. For recrystallisation solvents, mp, Rf-values (tlc), analyses and spectra see Tables II and III. Most of the solid BIM esters are soluble in DMF, chloroform and benzene, slightly soluble in ethanol and cyclohexane and insoluble in water and petrolium ether. BIM hexanoate **20f** (nmr and ms: Table III) precipitated as oil.

The following esters were prepared only (or additionally) on analytical scale according to the "Derivatization Procedure for the Fluorodensitometric Evaluation of Carboxylic Acids on Analytical Scale" (Rf values with benzene-ether 90:20 as elution solvent in brackets): BIM valerate **20e** (oily ester, 0.47), BIM hexanoate **20f** (0.49), BIM decanoate **20h** (0.53), BIM oleate **20m** (oily ester, 0.56), BIM (*p*-methoxybenzoate **20g** (0.44), BIM hydrogen oxalate **20t** (0.46, by-product of the preparation of **21a**), BIM hydrogen succinate **20u** (0.49, by-product of the preparation of **21b**), bis BIM oxalate **21a** (0.35).

BIM Stearate (**20k**). Example for the Preparation of BIM Esters **20** Directly from CMBI and Carboxylic Acids (Table II, III).

CMBI **19** (0.5 g, 2.3 mmoles), stearic acid (0.65 g, 2.3 mmoles), DMF (8 ml) and potassium hydrogen carbonate (2.3 g, 23 mmoles) are placed in an Erlenmeyer flask with calcium chloride tube and heated with stirring to 50° for 1 hour. After cooling **20k** is precipitated by addition of 10 ml of water in drops and recrystallized from ethanol to yield 0.95 g (88%) of **20k**, yellow needles, mp 58°. For elemental analysis and spectra see Table II and III.

BIM Methyl Ether (**22a**). Example for the Preparation of BIM Ethers **22** from CMBI and Excess of Alcanols (Table II, III).

CMBI (1 g, 4.6 mmoles) is dissolved in 4 g (125 mmoles) of hot methanol and the mixture is heated under reflux for 10 minutes. The hot solution is filtered and cooled. The precipitate obtained from the filtrate is filtered and the crystals are washed with methanol to give 0.8 g (82%) of **22a**, yellow platelets, mp 107°.

BIM ethyl ether **22b** was analogously prepared. **22a,b** (and **e**, see below) are soluble in DMF, chloroform and benzene, slightly soluble in methanol, ethanol and cyclohexane, and insoluble in water. For analyses and spectra see Table II and III.

BIM Stearyl Ether (**22e**). Example for the Preparation of BIM Ethers **22** from CMBI and Equimolar Amounts of Alcanols (Table II, III).

A solution of 0.62 g (2.3 mmoles) of stearyl alcohol and of 0.5 g (2.3 mmoles) of CMBI in 4 ml DMF is placed in an Erlenmeyer flask with reflux condenser and calcium chloride tube. Potassium hydrogen carbonate (0.46 g, 4.6 mmoles) is added and the mixture is heated with stirring to 100° and kept at this temperature for 20 minutes. After cooling, 10 ml of water is added drop by drop with vigorous stirring. The precipitate formed is filtered and recrystallized from ethanol to give 0.8 g (77%) of **22e**, yellow cubes, mp 66° (Table II and III).

BIM butyl ether **22c** and the BIM hexyl ether **22d**, which were analogously prepared, precipitated as oils. Their nmr spectra are nearly identical with the one of **22e** (Tables III), but the intensities of the signals for $(CH_2)_n$ at about 1.24 ppm are 2 and 6, respectively. BIM *sec*-butyl ether **22f** and BIM *t*-butyl ether **22g** were prepared only on analytical scale. The Rf values of these ethers (elution solvent: benzene-methanol-ether 90:5:10) are 0.62 (**22c**), 0.66 (**22d**), 0.63 (**22f**) and 0.61 (22 g).

1,3-Bis BIM-5-ethyl-5-phenylbarbituric Acid (**25**).

CMBI (0.5 g, 2.3 mmoles) and monosodium salt of 5-ethyl-5-phenylbarbituric acid (0.55 g, 2.3 mmoles) are heated in 8 ml of DMF as described for the preparation of ester **20b**. Water (10 ml) is added and the resulting precipitate is recrystallized from ethanol to give 0.55 g (80%) of **25**, yellow needles, mp 245°, tlc (e.s.I), Rf = 0.09; uv (chloroform): 252 (41580), 276 (11620), 322 (5960), 336 (8050), 360 (6470); ir (potassium bromide): 1720/1695 (amide I), 1495, 1435, 1293, 770 (CH-out of plane vibr); nmr (deuteriochloroform): (for the position of protons u-z see Scheme of Table III); δ 0.82 (t, CH_3, 3H), 2.35 (qua, CH_2, 2H, J = 7Hz), 6.12 and 6.22 (dd, 2 N-CH_2-N, equivalent AB systems, J = 15 Hz, 4H), 6.6-7.25 (2u, 2v and C_6H_5, 9H), 7.47 (t, 2w, $J_{7',6'} = J_{7',8'} = 8$ Hz), 7.68 (t, 2x, $J_{4',3'} = J_{4',5'} = 8$ Hz), 8.02 (d, 2y), 8.07 (d, 2z).

Anal. Calcd. for $C_{36}H_{26}N_4O_5$: C, 72.72; H, 4.41; N, 9.42. Found: C, 72.62; H, 4.95; N, 9.34.

Derivatization Procedure for the Fluorodensitometric Evaluation of Carboxylic Acids on Analytical Scale.

The fluorimetric measurements were carried out on a Perkin-Elmer Fluorometer MPF 44, equipped with a Hitachi Accessory and a Perkin-Elmer Recorder 023.

Materials and Reagents.

Acetone p.a. (Merck, Darmstadt), dried over molecular sieve; dibenzo-18-crown-6; anhydrous potassium hydrogen carbonate. The precoated Silicagel 60 tlc plates (Merck, Darmstadt) were washed twice in chloroform-methanol (1:3) before use.

Procedure.

The sample containing 50 pmoles to 50 nmoles of a fatty acid, dissolved in 10-20 μl of dry aceton, is transferred to a conical vial and treated with a 20 fold molar excess of CMBI (immediately dissolved in dry acetone before use), a 5 fold molar excess of dibenzo-18-crown-6 and an

about 30-50 fold amount of finely powdered potassium hydrogen carbonate. The final volume should be not more than 100 µl. The reaction mixture is heated to 50° and kept at this temperature for 15 minutes with shaking. After cooling, an aliquot of 2µl of the reaction mixture is transferred to the tlc plate with a microcap. Development is carried out with cyclohexane-chloroform-diethyl ether (20:20:80) as elution solvent. After evaporation of the solvent, the spots are scanned at an excitation wavelength of 365 nm and an emission wavelength of 480 nm.

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