Fibrin-Stabilizing Factor Inhibitors. 11. Monodansylated Weak Aliphatic Diamines

Christine Ljunggren, Kurt-Jürgen Hoffmann, Pål Stenberg, Uno Svensson, J. Lars G. Nilsson,*,†

Department of Organic Chemistry, Faculty of Pharmacy, University of Uppsala, S-751 23 Uppsala, Sweden

Ann Hartkoorn, and Ragnar Lundén

AB KABI, Fack, S-104 25 Stockholm, Sweden. *Received January 22, 1974*

A series of monodansyl derivatives of weak aliphatic diamines, *e.g.*, 3-azacadaverine, 3-oxacadaverine, and 3-thiacadaverine, has been prepared and tested as specific inhibitors of the fibrin-stabilizing factor (FSF). These and other compounds were prepared in an attempt to obtain inhibitors of the donor type with a low basic strength, in order to achieve an increased amount of un-ionized primary amine at the site of reaction. Several of these compounds were found to be potent inhibitors of FSF, while one substance, monodansylthiacadaverine, was found to be the most active inhibitor of the donor type so far described.

The final step in the coagulation process is the cross-linking of the monomeric fibrin. In the polymeric protein thus formed, the single fibrin units are bound together by covalent amide bonds between the ϵ-amino group of a lysine residue of one fibrin molecule and the γ-carbonyl group of a glutamine residue of another fibrin molecule. The polymerization is catalyzed by a transamidating enzyme, the fibrin-stabilizing factor (FSF, F XIII). Thrombin and Ca^{2+} are required for activating the enzyme. The mechanism for this enzymatic catalysis and the specific inhibition of the enzyme have recently been reviewed.[1-3]

We are currently trying to find specific FSF inhibitors which potentially could be used against thrombotic diseases by aiding the thrombolysis. Synthetic primary amines (pseudo-donors)[2-6] as well as thiol esters (pseudo-acceptors)[7,8] have been shown to inhibit the fibrin cross-linking. One of the most studied FSF inhibitors is monodansylcadaverine [*N*-(5-aminopentyl)-5-dimethylamino-1-naphthalenesulfonamide]. Studies concerning the structure-activity relationship of this type of inhibitor[2-5] have revealed that a potent inhibitor preferably should have the general structure $ArSO_2NH(CH_2)_5NH_2$, where a primary amino group is bound *via* five methylene groups and a sulfonamide moiety to an aromatic nucleus.

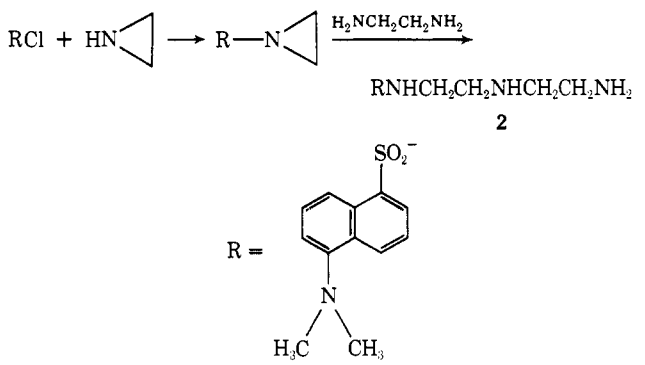
For small nonspecific pseudo-donors Lorand, *et al.*,[6] have published that the activity is highly dependent on the pK_a values of the primary amino group. In order to increase the total nucleophilic reactivity of the pseudo-donors at a fixed pH, we have now prepared compounds related to monodansylcadaverine where the primary amino group is affected by heteroatoms in the side chain. The compounds prepared and their activities are presented in Table I.

Chemistry. In the preparation of compounds 1, 3, and 4, the appropriate diamine was monoacylated[4] in chloroform using dansyl chloride (5-dimethylamino-1-naphthalenesulfonyl chloride) in the presence of *N*-methylpyrrolidine. Compound 2 was obtained as depicted in Scheme I; dansyl chloride was allowed to react with ethylenimine and the three-membered ring of the sulfonamide thus formed was opened by treatment with ethylenediamine.

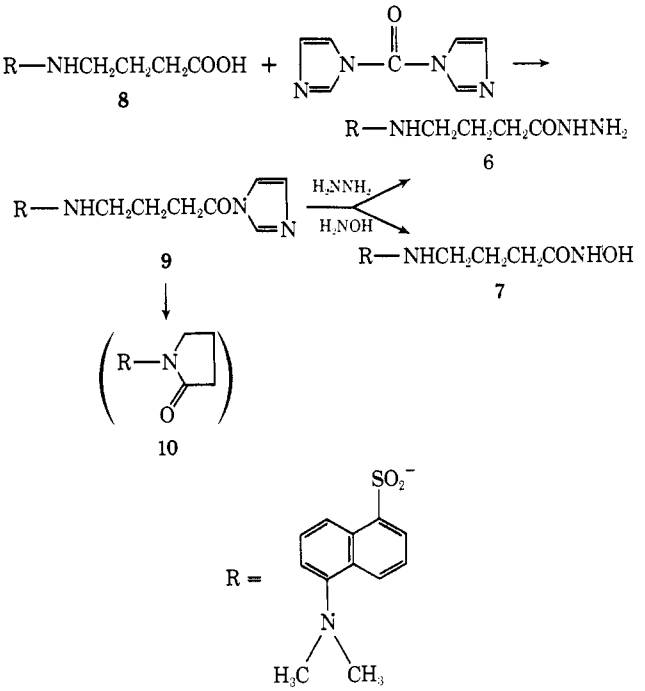
The glycine amide 5 was synthesized by reacting *N*-(2-aminoethyl)-5-dimethylamino-1-naphthalenesulfonamide[4] and glycine ethyl ester hydrochloride in DMF. In the preparation of 6 and 7, 4-(5-dimethylamino-1-naphthalenesulfonamido)butyric acid (8) (dansyl-GABA) was treated with *N,N'*-carbonyldiimidazole and the activated intermediate 9 (possibly 10)[9] reacted with hydrazine to form the hydrazide 6 or with hydroxylamine to form the hydroxamic acid 7 (Scheme II).

†Address correspondence to this author at AB Hässle, Fack, S-431 20 Mölndal, Sweden.

Scheme I



Scheme II



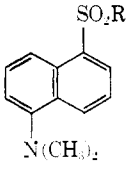
Bioassay. The biological assays were carried out using the clot lysis test previously described.[10] The inhibitory activity of each compound is expressed in per cent of that of monodansylcadaverine.[4]

Results and Discussion

The activities of the compounds are collected in Table I.

It has been proposed[6] that the fibrinoligase-catalyzed cross-linking of fibrin proceeds *via* an acyl enzyme intermediate, *viz.* a thiol ester between the γ-carboxyl group of

Table I. Physical Data and Inhibitory Activity of the Compounds Studied



Compd no.	R	Mp, °C	Yield, %	Derivative	Formula	Approx pK_a of primary amino group (ref)	Un-ionized amino group at pH 7.4,[a] %	Inhibitory act.[b]
1	$-NH(CH_2)_5NH_2$[c]					10.9 (11)	0.03	100
2	$-NHCH_2CH_2NHCH_2CH_2NH_2$	68–70	60	Base	$C_{16}H_{24}N_4O_2S$	8.6 (13)	5.9	12
3	$-NHCH_2CH_2OCH_2CH_2NH_2$	115–117	53	Base	$C_{16}H_{23}N_3O_3S$	9.6 (14)	0.63	150
4	$-NHCH_2CH_2SCH_2CH_2NH_2$	162–165	52	0.5 fumarate	$C_{16}H_{23}N_3O_2S_2 \cdot 0.5C_4H_4O_4$	9.6 (15)	0.63	610
5	$-NHCH_2CH_2NHCOCH_2NH_2$	Oil	34	Base	$C_{16}H_{22}N_4O_3S$	7.4 (6)	50	30
6	$-NHCH_2CH_2CH_2CONHNH_2$	Oil	55	Base	$C_{16}H_{22}N_4O_3S$	3.2[d]	99	22
7	$-NHCH_2CH_2CH_2CONHOH$	Oil	55	Base	$C_{16}H_{21}N_3O_4S$			8

[a] The pK_a of the primary amino group is estimated to be approximately the same as that of the unsubstituted diamine. [b] See ref 10. [c] Monodansylcadaverine. [d] C. R. Lindegren and C. Niemann, *J. Amer. Chem. Soc.*, **71**, 1504 (1949).

a glutamyl residue of fibrin and the HS- group of the active center cysteine of the enzyme. This intermediate may then react with the ϵ-amino group of lysine to form the intermolecular amide bond between the fibrin molecules. The cross-linking would thus occur *via* a nucleophilic attack by the primary amino group on the carbonyl group of the thiol ester. Similarly, monodansylcadaverine[4] probably inhibits the cross-linking when its primary amino group reacts with the acyl enzyme intermediate. For this reaction the H_2N- group must be present in its base form.

The bioassay of the inhibitors is performed at pH 7.4 in the test solution. The amount of monodansylcadaverine present in its un-ionized form at this pH can be calculated from the pK_a value of the primary amino group (the aromatic dimethylamino group is a much weaker base). This pK_a value is estimated to be 10.9 at 25°, the same as $pK_{a(1)}$ of cadaverine.[11] At a pH of 7.4, the cation of monodansylcadaverine would predominate to an extent of 99.97%. The situation may, however, be even more unfavorable since the pH at the site of action might be lower than that of the surrounding test solution. This has, *e.g.*, been found to be the case in the vicinity of some membranes.[12]

A lower pK_a value of the nucleophilic primary amino group of FSF inhibitors could thus markedly increase the activity by increasing the amount of the inhibitor present as the free base. Assuming that such weak amines would retain sufficient nucleophilicity, we have prepared four different inhibitors (**2**-**5**) including the monodansyl derivatives of 3-azacadaverine ($pK_{a(2)}$ at 30° = 8.6[13]), 3-oxacadaverine ($pK_{a(1)}$ at 30° = 9.6[14]), and 3-thiacadaverine ($pK_{a(1)}$ at 30° = 9.6[15]) (Table I).

We have previously demonstrated[4] that the length of the side chain in compounds related to monodansylcadaverine is of vital importance for the activity. In a homologous series of monodansyl derivatives of α,ω-diaminoalkanes, the activity has a maximum for monodansylcadaverine. Compounds **2**-**5** all have virtually the same length of the side chain. Two of these, **2** and **5**, are less active than **1**, while **3** and **4** are more active. Since the nucleophilicity is closely related to the basic strength of the amino group, it seems likely that the nucleophilicity of **2** and **5** is too low for the inhibiting reaction. It is also possible that the side chains of **2** and **5** are more extensively hydrated[16] than those of **3** and **4**. Besides, it is worth noticing that the secondary amino group of **2** is protonated to a large extent at pH 7.4 and consequently this compound has a positive charge in the middle of the side chain.

The high activity of **3** and **4** shows it is possible to increase the activity of inhibitors related to monodansylcadaverine by lowering the basic strength of the H_2N- group. The higher activity of **4** compared to **3** may indicate that the side chain of **4** is less hydrated[16] than that of **3**. Compound **4** (monodansylthiacadaverine) is the most active inhibitor of the donor type so far described. This compound may be used to increase the sensitivity of the various analytical test systems where monodansylcadaverine presently is used.[17-20]

A weak base is normally also a weak nucleophile. However, in compounds having an unshared electron pair on the atom next to the nucleophilic center the nucleophilicity normally is increased above what can be expected from their pK_a values (the α effect).[21-23] Applying this theory to our work we have prepared compounds **6** and **7** which both are very weak bases. These substances have significant FSF inhibitory properties although their activities are lower than that of monodansylcadaverine. Compound **7**, with an activity of 8% of that of monodansylcadaverine, is the first example of a pseudo-donor with a nucleophile other than an H_2N- group.

Experimental Section

General Comments. Melting points were determined with calibrated Anschütz thermometers in an electrically heated metal block. All crystalline compounds were characterized by elemental analyses (C, H, and N) which deviated maximally ±0.4% from the theoretical values. Ir spectra were run for identification purposes on a Perkin-Elmer 273 spectrophotometer and mass spectra were recorded with an AEI MS 30 apparatus at 70 eV. Dansyl chloride and *N,N'*-carbonyldiimidazole were commercially available.

***N*-(5-Amino-3-azapentyl)-5-dimethylamino-1-naphthalenesulfonamide (2, Scheme I).** To an ice-cold mixture of ethylenimine (0.9 g, 20 mmol), $Na_2CO_3 \cdot 10H_2O$ (10.0 g, 35 mmol), and 10 ml of water was added a solution of dansyl chloride (5.4 g, 20 mmol) in acetone (50 ml). The reaction mixture was kept at 10° during the addition of dansyl chloride whereupon it was stirred at room temperature for 30 min. It was then extracted with toluene (3 × 20 ml) and the organic phase was dried (Na_2SO_4). To the toluene solution was added ethylenediamine (1.2 g, 20 mmol); the mixture was kept at room temperature overnight and warmed to 50° for 2 hr. The solvent was evaporated and the residual oil was dissolved in 2 *M* HCl (100 ml) and washed with chloroform (2 × 100 ml). After adjusting the pH to 8 the aqueous layer was extracted with chloroform. The organic phase was washed several times with water whereupon it was dried (Na_2SO_4) and the solvent evaporated *in vacuo*, affording an oil which solidified upon standing: yield, 4.0 g (60%); mp 68–70° (from ligroine). *Anal.* ($C_{16}H_{24}N_4O_2S$) C, H, N.

***N*-(5-Amino-3-oxapentyl)-5-dimethylamino-1-naphthalenesulfonamide (3, Monodansyloxacadaverine).** To a solution of

N-methylpyrrolidine (0.6 g, 6.7 mmol) and bis(2-aminoethyl) ether (3-oxacadaverine)[24] (1.4 g, 13.4 mmol) in chloroform (5 ml) was slowly added dansyl chloride (1.8 g, 6.7 mmol) in chloroform (30 ml) at such a rate that the reaction mixture had a light green fluorescent color (about 2 hr). The precipitate formed was filtered off and washed with chloroform, and the combined filtrates were washed with saturated $NaHCO_3$ solution (2 × 150 ml) and with water (2 × 150 ml), dried (Na_2SO_4), and evaporated *in vacuo*, affording a light yellow crystalline residue: yield, 1.2 g (53%); mp 115–117° (from toluene). *Anal.* ($C_{16}H_{23}N_3O_3S$) C, H, N.

***N*-(5-Amino-3-thiapentyl)-5-dimethylamino-1-naphthalenesulfonamide (4, Monodansylthiacadaverine).** This compound was prepared from bis(2-amino ethyl) sulfide (3-thiacadaverine)[25,26] (8.9 g, 74.2 mmol) and dansyl chloride (10.0 g, 37.1 mmol) as described for **3**. The product, a fluorescent yellow oil, was dissolved in absolute ethanol (100 ml) and anhydrous ether (500 ml). A solution of fumaric acid in absolute ethanol was added and the precipitated fumarate was filtered off: yield, 9.0 g (59%); mp 162–165° (from 95% ethanol). *Anal.* ($C_{16}H_{23}N_3O_2S_2 \cdot 0.5C_4H_4O_4$) C, H, N.

***N*-(2-Glycinamidoethyl)-5-dimethylamino-1-naphthalenesulfonamide (5).** *N*-(2-Aminoethyl)-5-dimethylamino-1-naphthalenesulfonamide[4] (0.6 g, 2.5 mmol) and glycine ethyl ester hydrochloride (0.4 g, 2.8 mmol) in dimethylformamide (25 ml) were allowed to react at 100° for 4 hr. The solution was evaporated *in vacuo* and the residue was purified as described for compound **2** yielding 0.3 g (34%) of **5** as a yellow oil, identified by ir and mass spectra. Tlc showed only one spot: R_f 0.7 (silica gel G plates developed in ether–2% NH_3 in ethanol, 8:5); ν_{max} (film) 3500–3000 (-NH_2- and -NH-, br), 2780 [-$N(CH_3)_2$], 1670 and 1570 (-NHCO-), 1320 and 1140 cm^{-1} (-SO_2-); mass spectrum *m/e* (rel intensity) 335 (1, $M^+ - 15$), 321 (25), 293 (52), 250 (84), 235 (78), 171 (100), 170 (95), 169 (33), 168 (58).

4-(5-Dimethylamino-1-naphthalenesulfonamido)butyric Acid (8). Dansyl chloride (13.1 g, 48.5 mmol) was slowly added to a saturated $NaHCO_3$ solution (20 ml) containing 4-aminobutanoic acid (GABA) (5.0 g, 48.5 mmol) and the mixture was allowed to react overnight. After adding more ether a water-soluble precipitate was obtained. This was filtered off and the filtrate evaporated. The residue, dissolved in chloroform (200 ml), was extracted with saturated $NaHCO_3$ solution (2 × 150 ml). After adjusting the pH to 5 the aqueous layer was extracted with chloroform (3 × 150 ml). The organic phase was washed with water (3 × 300 ml), dried (Na_2SO_4), and evaporated *in vacuo*, affording an oil (5.5 g, 34%), which was identified as its hydrochloride, mp 183–185° dec (from acetone–ethanol–ether). *Anal.* ($C_{16}H_{20}N_2O_4S \cdot HCl$) C, H, N.

4-(5-Dimethylamino-1-naphthalenesulfonamido)butyric Acid Hydrazide (6, Scheme II). To a solution of *N,N'*-carbonyldiimidazole (0.5 g, 3.1 mmol) in dry THF (40 ml) was added 4-(5-dimethylamino-1-naphthalenesulfonamido)butyric acid (8, dansyl-GABA)[9] (1.0 g, 3.1 mmol) in dry THF (10 ml). (If the reaction mixture was worked up, dansyl-γ-butyrolactam[9] (**10**) was identified as the product.) After 2 hr at room temperature hydrazine hydrate (0.3 g, 6.2 mmol) was added.[27] The reaction mixture was stirred overnight, the solvent evaporated *in vacuo*, and the residue dissolved in chloroform. The organic layer was washed with water several times, dried (Na_2SO_4), and evaporated. The product, a yellow oil, was precipitated as its hydrochloride. This salt (0.5 g, 55%) was hygroscopic and consequently the compound was identified as the base by ir and mass spectra. Tlc (silica gel G plates developed in ethanol–ether 1:5) showed only one spot at R_f 0.9; ν_{max} (film) 3400–3000 (-NH_2- and -NH-), 2780 [-$N(CH_3)_2$], 1650 (>C=O), 1310 and 1140 cm^{-1} (-SO_2-); mass spectrum *m/e* (rel intensity) 350 (10, M^+), 335 (6), 318 (16), 207 (32), 171 (100), 170 (80), 169 (22).

***N*-(5-Dimethylamino-1-naphthalenesulfonamido)butyrylhydroxamic Acid (7, Scheme II).** This compound was prepared in the same way as compound 6. The substance was obtained as an oil (0.5 g, 55%). The tlc of the product showed only one spot (silica gel G plates developed in ethanol–ether 1;5), R_f 0.8. The compound was identified by ir and mass spectra: ν_{max} (film) 3400–3100 (br, -NH- and -OH), 2780 [-$N(CH_3)_2$], 1650 (>C=O), 1310 and 1140 cm^{-1} (-SO_2-); mass spectrum *m/e* (rel intensity) 351 (0.5, M^+), 335 (13), 318 (56), 317 (50), 171 (100), 170 (56), 169 (22), 168 (34).

Acknowledgments. We wish to thank Professor Laszlo Lorand, Northwestern University, for advice and discussions. This work has been supported by the Swedish Medical Research Council, Project No. 03X-3770.

References

(1) L. Lorand, *Thromb. Diath. Haemorrh., Suppl.*, **39**, 75 (1970).
(2) J. L. G. Nilsson, P. Stenberg, Ch. Ljunggren, K.-J. Hoffmann, R. Lundén, O. Eriksson, and L. Lorand, *Ann. N. Y. Acad. Sci.*, **202**, 286 (1972).
(3) L. Lorand and J. L. G. Nilsson. *Drug Des.*, **3**, 415 (1972).
(4) J. L. G. Nilsson, P. Stenberg, Ch. Ljunggren, O. Eriksson, and R. Lundén, *Acta Pharm. Suecica*, **8**, 497 (1971).
(5) P. Stenberg, Ch. Ljunggren, J. L. G. Nilsson, R. Lundén, and O. Eriksson, *J. Med. Chem.*, **15**, 674 (1972).
(6) L. Lorand, N. G. Rule, H. H. Ong, R. Furlanetto, A. Jacobsen, J. Downey, N. Öner, and J. Bruner-Lorand, *Biochemistry*, **7**, 1214 (1968).
(7) L. Lorand, C.-H. Chou, and I. Simpson, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2645 (1972).
(8) Ch. Ljunggren, K.-J. Hoffmann, P. Stenberg, and J. L. G. Nilsson, *J. Med. Chem.*, **16**, 1186 (1973).
(9) N. Seiler and J. Wiechmann, *Hoppe-Seyler's Z. Physiol. Chem.*, **350**, 1493 (1969).
(10) J. L. G. Nilsson, P. Stenberg, O. Eriksson, and R. Lundén, *Acta Pharm. Suecica*, **7**, 441 (1970).
(11) R. Barbucci, P. Paoletti, and A. Vacca, *J. Chem. Soc. A*, 2202 (1970).
(12) J. L. Kavanau, "Structure and Function in Biological Membranes," Vol. 2, Holden-Day, San Francisco, Calif., 1965, p 331.
(13) G. H. McIntyre, B. P. Block, and W. C. Fernelius, *J. Amer. Chem. Soc.*, **81**, 529 (1959).
(14) J. R. Lotz, B. P. Block, and W. C. Fernelius, *J. Phys. Chem.*, **63**, 541 (1959).
(15) E. Gonik, W. C. Fernelius, and B. E. Douglas, *J. Amer. Chem. Soc.*, **76**, 4671 (1954).
(16) J. Clark and D. D. Perrin, *Quart. Rev., Chem. Soc.*, **18**, 295 (1964).
(17) L. Lorand, T. Urayama, J. W. C. de Kiewiet, and H. L. Nossel, *J. Clin. Invest.*, **48**, 1054 (1969).
(18) L. Lorand, O. M. Lockridge, L. K. Campbell, R. Myhrman, and J. Bruner-Lorand, *Anal. Biochem.*, **44**, 221 (1971).
(19) L. Lorand and L. K. Campbell, *Anal. Biochem.*, **44**, 207 (1971).
(20) R. O. Morton, D. Bannerjee, R. Delaney, and J. W. Hampton, *Clin. Res.*, **18**, 613 (1970).
(21) J. D. Aubort and R. F. Hudson, *Chem. Commun.*, 937 (1970).
(22) G. Klopman, K. Tsuda, J. B. Louis, and R. E. Davis, *Tetrahedron*, **26**, 4549 (1970).
(23) G. Biggi and F. Pietra, *J. Chem. Soc. B*, 44 (1971), and references cited therein.
(24) V. M. Savinov and L. B. Sokolov, *Zh. Prikl. Khim. (Leningrad)*, **34**, 2124 (1961).
(25) A. Marxer and K. Miescher, *Helv. Chim. Acta*, **34**, 924 (1951).
(26) A. H. Nathan and M. T. Bogert, *J. Amer. Chem. Soc.*, **63**, 2363 (1941).
(27) H. A. Staab, M. Lüking, and F. H. Dürr, *Chem. Ber.*, **95**, 1275 (1962).