Substituted 3,4-Pentadienyldiamines as Inhibitors of Platelet Aggregation

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N-(2-Butyl-2-ethyl-5-methyl-3,4-hexadienyl)-1,3-propanediamine (**10**, RMI 10,277) was found to inhibit *in vitro* and *in vivo* ADP-induced platelet aggregation with minimal release of procoagulant platelet factor 3 (PF3). The diamines **26**, **30**, **39**, and **47** and the triamine **9** were also found to have high *in vitro* potency of about ten times that of previously known agents, excepting prostaglandin PGE_1. Structure-activity correlation indicated that a 3- to 4-carbon chain between the amino functions is optimal and that compounds with primary and secondary amino functions are more potent. Variation of the allenic portion of the molecule did not greatly affect activity. Compound **48** showed unusually high PF3 release and, as a consequence, shortened coagulation time. This demonstrated the importance of measuring the undesirable PF3 release caused by some platelet aggregation inhibitors. *In vivo* evaluation in guinea pigs in two test systems showed **10** to have oral activity at 10 and 30 mg/kg, respectively.

Blood platelets or thrombocytes have a number of physiologic functions that can be considered in three broad categories: they participate in the coagulation process; they serve as vehicles for biologically active substances; and they maintain the integrity of blood vessels.[1-3] Adhesion of platelets to a surface, as well as other stimuli, initiates a secretory process known as the release reaction. The contents of certain granules are expelled from the platelet into the plasma without disruption of the platelet membrane. The release reaction is followed by aggregation of adjacent platelets and formation of either a hemostatic plug or of a thrombus. In 1961, Gaarder, *et al.*,[4] demonstrated that adenosine diphosphate (ADP) produced platelet aggregation. Since then, many other substances have been found to do this but there is good evidence that most of these act indirectly *via* ADP. Several lines of evidence indicate that abnormal platelet functions are implicated in thrombosis.[5,6] Agents that would normalize abnormal platelet functions in the thrombosis-prone individual may have great therapeutic value. For this reason we have searched for agents that inhibit ADP-induced platelet aggregation. We reported earlier our findings on certain 2-piperidineethanols of benzyl- and benzylidenefluorene,[7-9] on certain lactamimides,[10] and on a member of a series of anilines[11] obtained from Zellner.[12] We now wish to report our finding on certain substituted 3,4-pentadienyldiamines.

An exploratory series of allene amines and diamines was prepared, prompted by the recent commercial availability of a stable allene aldehyde, 2-butyl-2-ethyl-5-methyl-3,4-hexadienal. Some of these were found to inhibit ADP-induced platelet aggregation. We then explored systematically the effect of structural modifications on this activity by preparing and evaluating the compounds listed in Table I.

Inhibition of *in Vitro* ADP-Induced Aggregation. This study revealed that activity is distributed broadly throughout the series, but that, unlike our previous experience in other series, we found in this series very pronounced changes in potency with relatively small structural changes. Thus, the optimal length of the chain A connecting the two amino groups consists of 3–4 carbon atoms; an increase from 2 to 3 carbon atoms (**1** *vs.* **12**; **2** *vs.* **14**; **6** *vs.* **16**; **27** *vs.* **34**) enhanced activity while further lengthening to 5 or 6 carbon atoms did not substantially increase inhibitory activity but enhanced release of PF3 (**12** *vs.* **19**, **20**). This is an undesirable effect (*vide infra*). Branching at the A chain by one methyl group did not greatly change activity (**14** *vs.* **25**) and cyclic forms **30** and **37–48** were among the most potent compounds, particularly the 4-piperidylmethyl- and -ethylamines **39** and **47**. On the other hand, geminal dimethyl groups (**21**, **22**, **24**) or carbocyclic branching as in **36** led to a sharp decrease in activity.

The nature of the $-NR^1R^2$ group influenced activity greatly. In the straight 3-carbon A-chain series, the primary amine **10** was superior to secondary amines **11**, **13**, and **16**, which in turn were superior to the corresponding tertiary amines **12**, **14**, and **17**, respectively, and to the heterocyclic amines **32–35**. Similarly, the unsubstituted 4-piperidylmethylamine **39** was superior to the substituted congeners **40–45**. Another heterocyclic amine, compound **30**, which contains a cyclic amidine function showed very high potency. Of a number of triamines (**7**, **9**, **26**, **35**, **48**, **49**), compounds **9** and **26** are interesting. Structural variation of the allene portion of the molecule (compounds, **49–60**) indicated that at least one terminal alkyl group is required (*cf.* **52**). Compared to **14**, compounds **54–56**, **59**, and **60** showed about equal activity while **53**, **57**, and **50** were less active.

Comparison to six reference compounds listed at the end of Table I showed that compounds **9**, **10**, **26**, **30**, **39**, and **47** were about ten times more potent than the first five. Prostaglandin PGE_1, which is the most potent known inhibitor of platelet aggregation, on the other hand, was again 10–30 times more active.

PF3 Release. Several of the compounds listed in Table I caused PF3 release or had PF3-like properties, as indicated in the last three columns of Table I. PF3 is a procoagulant factor that enhances coagulation and its release is an undesired property. We have shown earlier that a normal breakfast causes PF3-like activity in man of 0.1–0.3% and we adopted these values as our limit of acceptability.[13] The values reported in Table I are given in per cent of maximum available PF3 activity. Maximum PF3 activity was determined after sonication of platelet-rich plasma. Several of the compounds were evaluated for effects on prothrombin and partial thromboplastin time and were found to have essentially no effect. This finding indicated that soluble plasma clotting factors were not affected. Similarly, compounds **10**, **26**, and **30** caused only a slight prolongation of whole blood clotting time, as shown in Table II. On the other hand, **48**, which caused unusually high PF3 release, was found to shorten whole blood clotting time. That this effect is due to release

of a platelet factor is further demonstrated by measuring the effect of **48** on thrombin time in platelet-rich *vs.* platelet-poor plasma (Table III). These experiments demonstrate the importance of determining PF3 release by platelet aggregation inhibitors.

In Vivo **Evaluations.** Determination of platelet aggregation inhibition in animals after oral administration represents a major problem in the selection of agents for clinical evaluation. This problem is compounded by the now obvious fact that there are many and chemically quite different agents that affect platelet functions in *in vitro* systems. Although some agents can be distinguished by their mechanism of action,[3] principal determinants of therapeutic usefulness will most likely be oral activity and duration of action. We have developed two *in vivo* systems. One consists of administering test compound for several days to animals to obtain a blood sample 2 hr after the last dose and to prepare citrated platelet-rich plasma from it. The degree of aggregation induced by a certain amount of ADP is then determined *in vitro* and is compared to a value obtained at the same time from an untreated control group.[11] The other test system is entirely *in vivo* and consists of measuring blood platelet count after an ADP-infusion with and without prior treatment by test compound.[9] These test systems have been described previously and it was found that of a number of small animals, the guinea pig most closely resembles man in respect to ADP-induced platelet aggregation. Compounds **9**, **10**, and **39** were evaluated in the *in vivo-in vitro* system (Table IV). Compounds **9** and **10** were found to be more active than **39**. The effect of orally administered **10** on the decrease of platelet count upon ADP infusion is shown in Figure 1. ADP-induced aggregation is inhibited at 30 mg/kg po. Compound **10**, thus, showed oral activity in both systems.

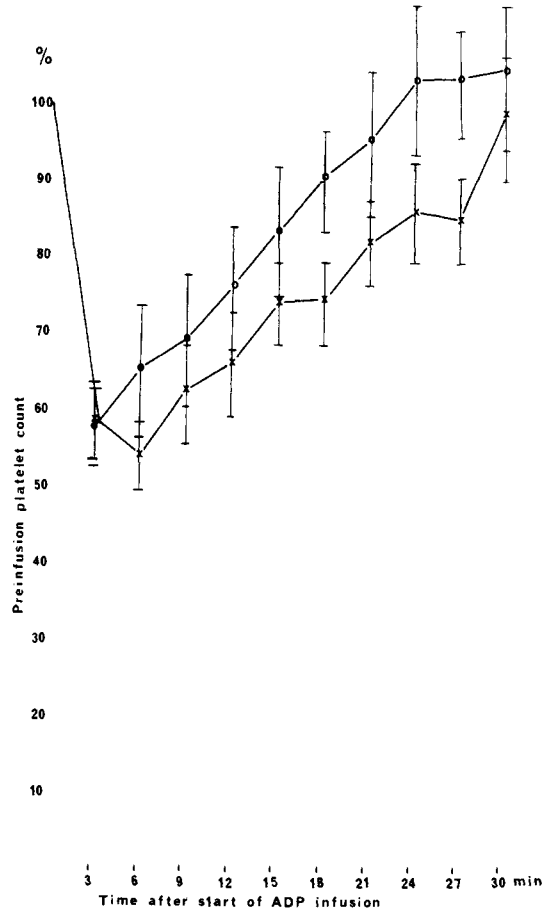
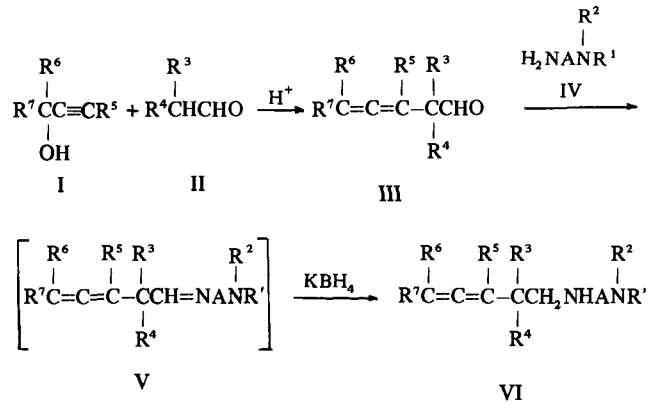


Figure 1. Effect of **10** on platelet aggregation in guinea pigs 2 hr after 30 mg/kg po: x, control group (six animals), 0.2 mg/kg of ADP infused over 1 min; o, treated group (six animals).

Chemistry. The substituted 3,4-pentadienyldiamines listed in Table I were prepared by condensation of an allene aldehyde III with a diamine IV, in which one of the amino functions was primary, and *in situ* reduction of the resulting Schiff base V with KBH_4 to give VI, as outlined in Scheme I.

Scheme I



The allene aldehydes were obtained from substituted propargyl alcohols I and aldehydes II by the method of Thompson.[14] This procedure was generally applicable and gave good yields as shown in Table I. Preparation of compounds **10** and **50** required the use of a bis-primary diamine IV (R^1R^2 = H) and a large excess of it was used to obtain the monosubstituted condensation product. Compound **24** was obtained by condensation of 2-butyl-2-ethyl-5-methyl-3,4-hexadienylamine with 3-diethylamino-2,2-dimethylpropionaldehyde. Seven allene aldehydes were obtained from Thompson,† while others were prepared by the same procedure and are listed in Table V. Most of the diamines were obtained from commercial sources;‡ 1-piperidinocyclohexylmethylamine was obtained by $LiAlH_4$ reduction of 1-piperidinocyclohexanecarboxamide. Typical examples for the synthetic procedures are given in the Experimental Section. The compounds listed in Tables I and V all exhibit the strong characteristic allene ir absorption in the 1930–1980-cm^{-1} region.[15,16]

Experimental Section §

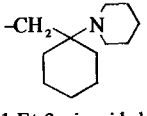
N-(2-Butyl-2-ethyl-5-methyl-3,4-hexadienyl)-*N'*,*N'*-diethyl-1,3-propanediamine (14). A mixture of 26 g (0.2 mol) of $Et_2N(CH_2)_3NH_2$ and 39 g (0.2 mol) of 2-butyl-2-ethyl-5-methyl-3,4-hexadienal in 200 ml of MePh was refluxed under a Dean-Stark trap until the theoretical amount of H_2O had collected (3.5 ml, 2 hr). The mixture was evaporated to dryness and the residue was added over 30 min to 16 g of KBH_4 under 200 ml of cold (15°) MeOH. After stirring overnight, the solvent was evaporated, 150 ml of H_2O was added, and the product was extracted into pentane. After evaporation of solvent, the product was distilled: 42 g (89%); bp 119–121° (0.28 mm); $n^{28}D$ 1.4695; ir (neat) 1962 cm^{-1} (Table I). This procedure was used for the preparation of most compounds listed in Table I.

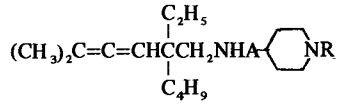
†We are grateful to Messrs. B. Thompson and J. P. Hill of Tennessee Eastman Co. for generous supplies of substituted 3,4-pentadienals.

‡We thank Dr. W. H. Rieger of Reilly Tar and Chemical Co. for intermediate piperidine derivatives, Mr. D. M. Hawley of The Dow Chemical Co. for 2-aminoethylaziridine, and Mr. J. R. Berg of Parke, Davis & Co. for piperidinocyclohexylcarboxamide.

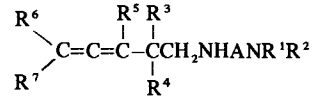
§See footnote *a*, Table I.

Table I. Substituted 3,4-Pentadienyldiamines as Inhibitors of Platelet Aggregation in Human Plasma

	A	R^1	R^2	Bp, °C (mm)	Formula[a]	% yield	Effects on human blood platelets[b]						
							Inhibn of aggregn, μg/ml				% PF3 release, μg/ml		
							100	30	10	3	300	100	30
					$(CH_3)_2C{=}C{=}CHCCH_2NHANR^1R^2$ (C4H9 = C_4H_9, C2H5 = C_2H_5)								
1	$(CH_2)_2$	Me	Me	96–98 (0.25)	$C_{17}H_{34}N_2$	53	85	16				0.015	
2	$(CH_2)_2$	Et	Et	117–119 (0.35)	$C_{19}H_{38}N_2$	80	93	41					
3	$(CH_2)_2$	*i*-Pr	*i*-Pr	127–128 (0.30)	$C_{21}H_{42}N_2$	68	50	15					
4	$(CH_2)_2$	Pr	Pr	124–125 (0.30)	$C_{21}H_{42}N_2$[c]	62	28						
5	$(CH_2)_2$	Et	Bu	122–125 (0.25)	$C_{21}H_{42}N_2$	69	64						
6	$(CH_2)_2$	H	$(CH_2)_2OH$	144–146 (0.30)	$C_{17}H_{34}N_2O$	53	100	54	18		0.51	0.081	0.018
7	$(CH_2)_2$	H	$(CH_2)_2NEt_2$	145–148 (0.35)	$C_{21}H_{43}N_3$	67	100	58	5			0.31	0.048
8	$(CH_2)_2$	H	CH_2-4-pyridyl	174–175 (0.30)	$C_{21}H_{35}N_3$	55	37						
9	$(CH_2)_2$	H	CH_2-4-piperidyl	160–162 (0.20)	$C_{21}H_{41}N_3$	50	100	100	86 (5)	4 (4)		1.20	0.014
10	$(CH_2)_3$	H	H	110–111 (0.25)	$C_{16}H_{32}N_2$	50	100	100	80 (2)	19 (2)[d]	3.2	0.38 (3)	0.031 (3)
11	$(CH_2)_3$	H	Me	105–107 (0.15)	$C_{17}H_{34}N_2$	61	100	100	66 (2)	13 (2)	4.3	0.25	0.081
12	$(CH_2)_3$	Me	Me	114–116 (0.40)	$C_{18}H_{36}N_2$	68	100	99 (2)	45 (2)	0 (2)		0.45 (2)	0.155 (2)
13	$(CH_2)_3$	H	Et	111–113 (0.30)	$C_{18}H_{36}N_2$	50	100	98	43	0		0.330	0.120
14	$(CH_2)_3$	Et	Et	119–121 (0.28)	$C_{20}H_{40}N_2$	89	100	72 (6)	33 (6)			0.057	0.020
15	$(CH_2)_3$	Bu	Bu	146–148 (0.20)	$C_{24}H_{48}N_2$	60	72	0					
16	$(CH_2)_3$	H	$(CH_2)_2OH$	156–158 (0.30)	$C_{18}H_{36}N_2O$	45	100	89	36			0.28	0.064
17	$(CH_2)_3$	$(CH_2)_2OH$	$(CH_2)_2OH$	170–171 (0.10)	$C_{20}H_{40}N_2O_2$	18		41	0				
18	$(CH_2)_3$	H	Cyclohexyl	150–152 (0.30)	$C_{22}H_{42}N_2$	77	100	72	1		0.95	0.68	0.060
19	$(CH_2)_5$	Me	Me	126–217 (0.35)	$C_{20}H_{40}N_2$	77	100	100	44 (3)	5 (2)		1.0	0.380
20	$(CH_2)_6$	Me	Me	134–136 (0.35)	$C_{21}H_{42}N_2$	66	100	100	36 (3)	10 (3)		1.50	0.550
21	CMe_2CH_2	H	*i*-Pr	110–112 (0.20)	$C_{20}H_{40}N_2$	74	93	24				0.026	
22	CMe_2CH_2	H	CMe_2CH_2OH	134–137 (0.25)	$C_{21}H_{42}N_2O$	55		15					
23	$CH_2CHOHCH_2$	Et	Et	140–142 (0.28)	$C_{20}H_{40}N_2O$	73	100	38 (2)	19 (2)	5	0.21	0.095 (2)	0.048 (2)
24	$CH_2CMe_2CH_2$	Et	Et	133–135 (0.20)	$C_{22}H_{44}N_2$	48		2				0.088	0.023
25	$CHMe(CH_2)_3$	Et	Et	132–134 (0.25)	$C_{22}H_{44}N_2$	77	100	87 (2)	40 (2)	8 (2)		0.660	0.160
	A	$-NR^1R^2$											
26	$(CH_2)_2$	1-Piperazinyl		130–133 (0.20)	$C_{19}H_{37}N_3$	67	100	97	69 (3)	0	0.566 (3)	0.720 (5)	0.343 (3)
27	$(CH_2)_2$	Morpholino		136–137 (0.30)	$C_{19}H_{36}N_2O$	60	50						
28	$(CH_2)_2$	1-Pyrrolidinyl		125–126 (0.30)	$C_{19}H_{36}N_2$	67		37					
29	$(CH_2)_2$	1-Aziridinyl		105–108 (0.45)	$C_{17}H_{32}N_2$	41	34	22					
30	$(CH_2)_2$	2-Me-1-imidazolidyl		144–146 (0.20)	$C_{19}H_{35}N_3$	51	100	100	47 (2)	26 (3)	2.50 (2)	0.360	0.069
31	$(CH_2)_2$	1-Me-2-pyrrolidinyl		122–125 (0.20)	$C_{20}H_{38}N_2$	66	100	76	53	2			
32	$(CH_2)_3$	Piperidino		135–137 (0.30)	$C_{21}H_{40}N_2$	73	100	88	15				
33	$(CH_2)_3$	4-Pr-piperidino		150–153 (0.20)	$C_{24}H_{46}N_2$	73	72	5					
34	$(CH_2)_3$	Morpholino		135–138 (0.15)	$C_{20}H_{38}N_2O$	74	71	21	17				
35	$(CH_2)_3$	4-Me-1-piperazinyl		137–138 (0.20)	$C_{21}H_{41}N_3$	61	100	74	0			0.76	0.120
36				154–157 (0.15)	$C_{25}H_{46}N_2$	65	0						
37		1-Et-3-piperidyl		131–132 (0.22)	$C_{28}H_{38}N_2$	70	38	1					
38		2,2,6,6-Me_4-4-piperidyl		122–124 (0.20)	$C_{22}H_{42}N_2$	77	100	16	10			0.75	0.050



	A	R									
39	CH_2	H	137–138 (0.25)	$C_{19}H_{36}N_2$	69	100	100	52 (2)	13 (3)	0.606 (3)	0.134 (4)
40	CH_2	Me	128–129 (0.35)	$C_{20}H_{38}N_2$	66		75	2			
41	CH_2	Bu	145–146 (0.20)	$C_{23}H_{44}N_2$	66	100	45	4			
42	CH_2	Allyl	140–142 (0.20)	$C_{22}H_{40}N_2$	65	100	38	16			
43	CH_2	Benzyl	182–185 (0.20)	$C_{26}H_{42}N_2$	53	54	9				
44	CH_2	$(CH_2)_2OH$	157–159 (0.20)	$C_{21}H_{40}N_2O$	65	100	57	11			
45	CH_2	$(CH_2)_2CN$	173–174 (0.20)	$C_{22}H_{39}N_3$	62	73					
46	CHMe	Et	136–138 (0.20)	$C_{22}H_{42}N_2$[e]	75	100	70	12			
47	$(CH_2)_2$	H	140–143 (0.20)	$C_{20}H_{38}N_2$	75	100	100	84	21	0.620	0.075
48	$(CH_2)_2$	$(CH_2)_2$-4-piperidyl	195–199 (0.20)	$C_{27}H_{51}N_3$	40	100	100	78 (2)	12	7.1	6.1 (2)	1.550 (2)



	A	R^7	R^6	R^5	R^4	R^3	$-NR^1R^2$									
49	$(CH_2)_2$	Me	Me	H	Me	Me	1-Piperazinyl	106–108 (0.30)	$C_{15}H_{29}N_3$	64	80	49		0.07	0.010	<0.001
50	$(CH_2)_2$	Me	Hexyl	H	Me	Me	NH_2	110–114 (0.30)	$C_{16}H_{32}N_2$[f]	50		71	17		0.081	0.021
51	$(CH_2)_3$	H	Pr	H	Me	Me	NEt_2	109–111 (0.30)	$C_{17}H_{34}N_2$	77	100	29				
52	$(CH_2)_3$	H	H	Me	Et	Bu	NEt_2	115–117 (0.20)	$C_{19}H_{38}N_2$	18		0				
53	$(CH_2)_3$	Me	Et	H	Me	Me	NEt_2	105–107 (0.30)	$C_{17}H_{34}N_2$	83	100	47	16		0.260	0.095
54	$(CH_2)_3$	Me	Hexyl	H	Me	Me	NEt_2	128–130 (0.25)	$C_{21}H_{42}N_2$	81	100	94 (2)	19 (2)	7	1.965 (2)	0.569 (2)	0.155 (2)
55	$(CH_2)_3$	Me	Et	H	CH=CHEt	Et	NEt_2	120–121 (0.15)	$C_{21}H_{40}N_2$	88	100	86 (2)	31 (2)	2	2.1	0.190	0.075
56	$(CH_2)_3$	Me	$(CH_2)_2CH=CH_2$	H	Et	Bu	NEt_2	143–144 (0.25)	$C_{23}H_{44}N_2$	69	100	94 (2)	28 (2)	10	1.04	0.160	0.050
57	$(CH_2)_3$	Me	Me	H	*g*	*g*	NEt_2	140–142 (0.20)	$C_{28}H_{34}N_2$[h]	45	100	48	4		0.23	0.037	0.006
58	$(CH_2)_3$	Me	Me	H	*i*	*i*	NEt_2	117–120 (0.18)	$C_{19}H_{34}N_2$	63		38	0				
59	$(CH_2)_3$	*j*	*j*	H	*i*	*i*	NEt_2	145–146 (0.15)	$C_{22}H_{38}N_2$	78	100	96 (2)	34 (2)	0		0.56	0.100
60	$(CH_2)_3$	*j*	*j*	H	Et	Bu	NEt_2	150–153 (0.20)	$C_{23}H_{44}N_2$	59	100	96 (2)	23	2		0.53	0.120
α-[*p*-(Fluoren-9-ylidenemethyl)phenyl]-2-piperidineneethanol glycolate (RMI 10,393)[k]											99 (4)	28 (6)	1 (6)		0.747 (6)	0.162 (6)	0.003 (7)
Hexahydro-2-[1-(1-naphthyl)ethylimino]azepine·HCl (RMI 7822)[l]											70 (2)	46 (2)	0 (2)		0.037	0.003	
N-(2-Diethylaminoethyl)-*N*-(2-hydroxy-2-phenylethyl)-2,5-dichloroaniline (AN 162)[m]											62 (118)	10 (104)			0.52 (42)	0.100 (47)	
2,4,6-Trimorpholinopyrimido[5,4-*d*]pyrimidine (RA 433 BS)[n]											47 (2)	25	15		<0.001	<0.001	
2-(5,10-Dihydrothiazolo[3,2-*b*][2,4]benzodiazepin-3-yl)phenol·HBr[o]											85	45			<0.001	<0.001	
Prostaglandin PGE_1[p]											73 (3)	32 (5)	10 (3)				

[a] C, H, and N analyses were found within ±0.4% of calculated values except where indicated. [b] *In vitro* effect of test compound on the inhibition of platelet aggregation caused by adenosine diphosphate in human platelet-rich plasma. PF3 activity is given as per cent of maximum. Number in parentheses indicates number of determinations. [c] *Anal.* Calcd: C, 78.18; N, 8.69. Found: C, 77.77; N, 9.17. [d] 3 μg/ml of 10 corresponds to a molar concentration of 1.19×10^{-5} *M*. [e] *Anal.* C: calcd, 78.98; found, 78.53. [f] *Anal.* N: calcd, 11.10; found, 10.50. [g] R^3–C–R^4 together form bicyclo[2.2.1]-5-heptenylidene. [h] *Anal.* N: calcd, 9.26; found, 8.53. [i] R^3–C–R^4 together form 3-cyclohexenylidene. [j] R^6–C–R^7 together form c-C_6H_{10}=. [k] References 7 and 8. [l] Reference 10. [m] Reference 11. [n] H. Scheffler and J. Roch, German Patent 1,470,341 (1960) [*Chem. Abstr.*, 66, 55521 (1969)]; R. S. Elkeles, J. R. Hampton, A. J. Honour, J. R. A. Mitchell, and J. S. Pritchard, *Lancet*, 2, 751 (1968). [o] E. F. Elslager, *et al.*, *J. Med. Chem.*, 14, 397 (1971). [p] At concentration × 10^{-3}.

Table II. Effects on Whole Blood Clotting Time in Rats[a]

Compd no.	Dose, mg/kg	Route (no. of animals)	Time after administrn, hr	Whole blood clotting time in rats ± SEM, sec	Max effect, % of control
10	10	sc (5)	0	391 ± 10	
			2	435 ± 50	
			5	528 ± 50	130[b]
26	30	sc (5)	0	345 ± 19	
			1	490 ± 22	132[c]
			4	370 ± 33	
30	30	po (5)	0	430 ± 11	
			2	590 ± 53	
			5	645 ± 24	132[c]
48	30	sc (6)	0	435 ± 9	
			1	275 ± 25	
			4	220 ± 21	61[c]

[a]For method see Experimental Section and ref 11. [b]$p \leq 0.05$. [c]$p \leq 0.01$.

Table III. Shortening of Thrombin Time by 48[a]

Concn, μg/ml	Thrombin time, % of control	
	PRP	PPP
0	100	100
30	70	83
100	43	66
300	52	93

[a]Samples were incubated for 15 min at 37° and were clotted with purified thrombin (0.0626 units per milliliter of plasma). PRP = platelet-rich plasma, PPP = platelet-poor plasma as described in the Experimental Section. Values given are averages of duplicate determinations.

N-(2-Butyl-2-ethyl-5-methyl-3,4-hexadienyl)-1,3-propanediamine (10). To a refluxing solution of 64 g (0.8 mol) of $H_2N(CH_2)_3NH_2$ in 250 ml of MePh was added 46 ml (0.2 mol) of 2-butyl-2-ethyl-5-methyl-3,4-hexadienal over a period of 4 hr, while H_2O that formed was collected in a Dean-Stark trap. After the theoretical amount of H_2O had collected (2 hr of additional refluxing), the mixture was evaporated to dryness and the residue was added to 16 g of KBH_4 in cold MeOH. The product was isolated as described in the preceding paragraph and had the properties given in Table I.

N-(2-Butyl-2-ethyl-5-methyl-3,4-hexadienyl)-*N'*,*N'*-diethyl-2,2-dimethyl-1,3-propanediamine (24). A mixture of 20 g (0.1 mol) of 2-butyl-2-ethyl-5-methyl-3,4-hexadienylamine and 16 g (0.1 mol) of 3-diethylamino-2,2-dimethylpropionaldehyde in MePh was refluxed under a Dean-Stark trap and reduced as described above to give 24 (Table I).

1-[2-(2-Butyl-2-ethyl-5-methyl-3,4-hexadienylamino)ethyl]aziridine (29). A mixture of 86 g (1 mol) of *N*-(2-aminoethyl)aziridine and 230 ml (1 mol) of 2-butyl-2-ethyl-5-methyl-3,4-hexadienal in 1 l. of MePh was refluxed under a Dean-Stark trap for 3 hr (18 ml of H_2O collected). The mixture was evaporated to dryness and the residue was added to 80 g of KBH_4 in 1 l. of MeOH with cooling. The mixture was then refluxed for 18 hr and the product was obtained as described above to give 29 (Table I).

2-(3-Methyl-1,2-butadienyl)-5-norbornene-2-carboxaldehyde (63). A mixture of 29 ml of 3-methyl-1-butyn-3-ol, 27 g (0.3 mol) of 5-norbornene-2-carboxaldehyde, 25 ml of xylene, 25 ml of C_6H_6, 0.1 g of hydroquinone, and 4 drops of Ultra Tx-acid (a liquid mixture of aromatic sulfonic acids) was refluxed for 20 hr under a Dean-Stark trap. Approximately 5.4 ml of H_2O was collected. The mixture was washed with 20 ml of saturated $NaHCO_3$ solution and the organic layer was distilled to give 7 g (12%) of 63, bp 116–118° (0.3 mm), Table V. This material was used to prepare 57.

1-Piperidinocyclohexanemethylamine. To a stirred mixture of 8 g of $LiAlH_4$ in 280 ml of dry THF was added 28 g (0.133 mol) of 1-piperidinocyclohexanecarboxamide‡ in small portions and the mixture was stirred overnight at room temperature. After decomposition with 30 ml of a saturated Na K tartrate solution, collection of the precipitate by filtration, and evaporation of solvent of the filtrate, the product was distilled: 18 g (69%); bp 85–90° (0.5 mm). *Anal.* ($C_{12}H_{24}N_2$) C, H, N. This material was used to prepare 36.

2-Butyl-2-ethyl-5-methyl-3,4-hexadienylamine. To 70 g of $H_2NOH \cdot HCl$ in 500 ml of 85% aqueous MeOH was added a solution of 60 g of KOH in 30 ml of H_2O and, over 30 min, a solution of 230 ml (1 mol) of 2-butyl-2-ethyl-5-methyl-3,4-hexadienal in 500 ml of MeOH, and the mixture was refluxed overnight. After evaporation of MeOH, the oxime was extracted into pentane and distilled: 176 g (84%); bp 112–113° (0.25 mm). *Anal.* ($C_{13}H_{23}NO$) C, H, N.

A solution of 165 g (0.8 mol) of the oxime in 165 ml of THF was added over 8 hr to 40 g of $LiAlH_4$ in 900 ml of refluxing THF. The mixture was stirred at room temperature overnight. After decomposition with 200 ml of a saturated Na K tartrate solution, collection of the precipitate by filtration, and evaporation of solvent of the filtrate, the product was distilled: 130 g (83%); bp 75–78° (0.18 mm). *Anal.* ($C_{13}H_{25}N$) C, H, N. This material was used to prepare 24.

Biological Methods. Blood Collection and Isolation of Plasma. Whole blood was obtained from voluntary, experienced donors before breakfast. Donors were instructed to take no drugs, specifically aspirin, for 5 days before giving blood. If the plasma was lipidemic or, in a preliminary aggregation experiment, showed no second-phase aggregation (aspirin-like effect), this plasma was not used. Blood was collected by the two-syringe technique. It was decalcified with 3.8% Na citrate solution, one part to nine parts of blood. The citrated blood was centrifuged at 100g for 10 min and citrated platelet-rich plasma (PRP) was isolated. Platelet-poor plasma (PPP) was isolated by recentrifuging the blood residue at 1500g for 15 min.

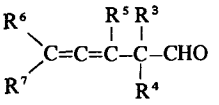
Inhibition of ADP-Induced Platelet Aggregation. Compounds were tested for inhibition of ADP-induced aggregation in a Bryston platelet aggregometer by the procedure of Mustard, *et al.*[17] Human platelet-rich plasma (PRP) was diluted with autologous platelet-poor plasma (PPP) to 400,000 platelets/mm³. Aqueous solutions of the test compound were prepared by addition of 1 equiv of 1 *N* HCl and were added to PRP to obtain the indicated concentrations. Saline was added to another sample of the same plasma to serve as control. After incubation for 20 min at 37°, ADP (2 μg/ml final concentra-

Table IV. Effect of Oral Administration to Guinea Pigs on *in Vitro* ADP-Induced Platelet Aggregation[a]

Compd no.	Daily dose (4 days), mg/kg po	No. of animals treated (control)	Concn of ADP, μg/ml PRP	Inhibition of aggregation					
				Av ΔT (% ± SEM)		Av total response (cm² ± SEM)			
				Control	Treated	Control	Treated	*P* value	% inhibn
10	10	7 (6)	0.45	21.8 ± 5.1	17.4 ± 2.8	4.56 ± 1.75	2.47 ± 1.06	<0.4	46
	10	7 (6)	0.80	34.8 ± 5.3	32.4 ± 2.8	7.06 ± 1.38	7.09 ± 0.56	N.S.	0
	30	3 (4)	0.45	15.2 ± 3.9	4.0 ± 6.4	1.03 ± 0.44	0.17 ± 0.09	<0.05	83
	30	3 (4)	0.80	36.7 ± 5.3	25.0 ± 10.4	7.17 ± 1.90	4.98 ± 3.64	<0.4	30
	30	7 (5)	0.80	38.6 ± 2.8	21.1 ± 5.0	6.15 ± 1.49	3.25 ± 1.59	<0.05	47
	100	Lethal							
9	30	5 (6)	0.45	38.0 ± 2.3	17.4 ± 4.5	6.85 ± 1.00	1.88 ± 1.25	<0.01	72
	30	5 (6)	0.80	58.3 ± 2.7	45.0 ± 3.1	11.93 ± 0.39	8.75 ± 0.67	<0.01	26
39	30	5 (6)	0.45	15.2 ± 3.4	10.6 ± 4.4	2.46 ± 1.20	1.88 ± 1.65	N.S.	23
	30	6 (6)	0.80	21.5 ± 2.3	23.2 ± 3.4	3.27 ± 0.75	3.34 ± 0.94	N.S.	0

[a]Compound administered for 4 days. Blood taken 2 hr after the last dose. See Experimental Section and ref 11.

Table V. Substituted 3,4-Pentadienals



No.	R^7	(R^6R^7C)	R^6	R^5	R^4	(R^3R^4C)	R^3	Bp, °C (mm)	Formula[a]	% yield
61	$CH_2=CH(CH_2)_2$		Me	H	Et		Bu	96–99 (0.15)	$C_{16}H_{26}O$[b]	63
62	Me		Me	H				65–68 (0.20)	$C_{12}H_{16}O$	63
63	Me		Me	H				116–118 (0.30)	$C_{13}H_{16}O$	12
64				H	Et		Bu	96–99 (0.10)	$C_{16}H_{26}O$	33
65				H				96 (0.15)	$C_{15}H_{20}O$	58

[a] C and H analyses were found within ±0.4% of calculated values unless indicated. [b] *Anal.* C: calcd, 82.00; found, 82.51.

tion) was added to induce aggregation. Platelet aggregation produces an increase in light transmittance (ΔT) through the plasma sample in the aggregometer and this response was recorded. The maxima of the ΔT responses for control and test sample were then used to calculate per cent inhibition of platelet aggregation by the test compound. More detail on the method and its variability is discussed elsewhere.[8,11]

Platelet Factor 3 Activation. Test compound solution was added to human citrated PRP and incubated at 37° for 20 min; a modified Stypven test was then performed. Plasma was diluted 1:10 for this modified test.[13]

Whole Blood Clotting Time. Whole blood clotting time was determined by the siliconized capillary method described elsewhere.[11] Blood samples were taken from the tail vein of rats.

In Vivo **Effect on** *in Vitro* **ADP-Induced Aggregation.**[11] Test compound was given to guinea pigs by a stomach tube at the indicated dose for 4 days. An untreated control group was maintained alongside. Blood was removed by heart puncture 2 hr after the last dose and citrated PRP was isolated and adjusted for *in vitro* ADP-induced platelet aggregation. ADP was added at the concentration indicated in Table IV. Max ΔT were obtained as described above. Total response was obtained by measuring the area between aggregation curve and base-line transmittance for the 5-min period following ADP addition with a planimeter. Per cent inhibition was calculated from the average total response of treated *vs.* control group.

In Vivo **ADP Infusion in Guinea Pigs.** Animals were dosed by a stomach tube and 2 hr later were infused with ADP according to the method of MacKenzie, *et al.*[9] For the experiment depicted in Figure 1, ADP was infused over 1 min at a level of 0.2 mg/kg. Platelet concentrations were determined at 3-min intervals by a Coulter counter.

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