4) Influence of buffer content on the fluorescence intensity is less.
5) Existence of excess magnesium does not cause a decrease of fluorescence intensity as shown in the previous paper.[2]

This method is as high sensitive as atomic absorption spectrophotometry and is remarkably more excellent than the Oxin Method used previously in regard to sensitivity and precision.

[Chem. Pharm. Bull. 21(5)1151—1155(1973)]

UDC 615.273.5.015.11

Platelet Aggregation Inhibitors. V.[1] Pyrimidine Derivatives, Indole Derivatives, Benzothiophenes, and Benzoquinolizine Derivative

KIYOMI KIKUGAWA and MOTONOBU ICHINO

Tokyo Research Laboratory, Kohjin Co., Ltd.[2]

(Received November 13, 1972)

Predominance of platelets in the white clot of blood in arteries has focused attention of their importance in arterial occlusion and has suggested that the inhibitor of platelet aggregation may be more useful than standard anticoagulant therapy.[3] Several organic compounds that powerfully inhibit adenosine 5'-diphosphate- and/or collagen-induced platelet aggregation have been investigated,[4] and heterocyclic compounds including pyrimidopyrimidines,[5] thieno compounds,[6,7] thiazolo compounds,[8,9] benzonaphthyridine,[10] pyrimidines,[11] fluoren compound,[12] aniline derivative,[13] quinazoline,[14] and others[15] are known as strong inhibitors

1) Part IV: K. Kikugawa, K. Iizuka, and M. Ichino, *J. Med. Chem.*, **16**, 358 (1973).
2) Location: *Komiya-chō 51, Hachiōji-cyty, Tokyo.*
3) *a*) J.F. Mustard, M.F. Glynn, F. Hovig, L. Jorgensen, M.A. Packham, F. Nishizawa, and H.C. Rowsell, "Physiology of Hemostasis and Thrombosis," S.A. Johnson, W.H. Seegers, ed., Thomas Springfield, I11, 1966, p. 288; *b*) G.W. Schnetzer, *Amer. Heart J.*, **83**, 552 (1972).
4) J.F. Mustard and M.A. Packham, *Pharmacol. Rev.*, **22**, 97 (1970).
5) *a*) P.R. Emmons, M.J.G. Harrison, A.J. Honour, and J.G.R. Mitchell, *Nature* (London), **208**, 255 (1965); *b*) A.A. Hassanein, A.G.G. Turpie, G.P. McNicol, and A.S. Dorglas, *Brit. Med. J.*, **5**, 83 (1970).
6) M. Nakanishi, H. Imamura, and K. Goto, *Yakugaku Zasshi*, **90**, 564 (1970).
7) *a*) German Patent, 1940572 (Dr. Karl Thomae, G.m.b.H.); *b*) German Patent, 2032686 (Dr. Karl Thomae, G.m.b.H.); *c*) U.S. Patent, 3475429 (Boehringer Ingelheim); *d*) J.J. Sixma and A.M.C. Treeschnigg, *Scand. J. Haemat.*, **8**, 417 (1971).
8) E.F. Elslager, N.F. Haley, J.R. McLean, S.C. Perricone, D. Potoczak, H. Veloso, D. Worth, and R.H. Wheelock, *J. Med. Chem.*, **14**, 397 (1971).
9) *a*) Belgian Patent, 755404 (Sandoz, S.A.); *b*) Japanese Patent, Kokai, 784 (1972).
10) H. Ott and G.M. Smith, *Brit. J. Pharmacol.*, **43**, 460 (1971).
11) German Patent, 2149249 (Pfizer Inc.).
12) *a*) R.D. Mackenzie, T.R. Blohm, E.M. Auxier, J.G. Henderson, and J.M. Steinbach, *Proc. Soc. Exp. Biol. Med.*, **137**, 662 (1971); *b*) G.P. Claxton, J.M. Grisar, E.M. Roberts, and R.W. Fleming, *J. Med. Chem.*, **15**, 500 (1972).
13) E. Deutsh, K. Lechner, K. Moser, and L. Stockinger, *Thromb. Diath. Hemorrh.*, **26**, 145 (1971).
14) German Patent, 2152521 (Bristol Myers Co.).
15) *a*) E.F. Elslager, N.F. Haley, J.R. McLean, S.C. Perricone, D. Potoczak, H. Veloso, D.F. Worth, and R.H. Wheelock, *J. Med. Chem.*, **14**, 782 (1971); *b*) E.F. Elslager, N.F. Haley, J.R. McLean, D. Potoczak, H. Veloso, and R.H. Wheelock, *J. Med. Chem.*, **15**, 61 (1972).

of platelet aggregation. The present communication deals with the *in vitro* test result of a series of heterocyclic compounds such as pyrrolopyrimidines, pyrimidines, thienopyrimidines, indol derivatives, benzothiophenes, and benzoquinolizine as inhibitors of platelet aggregation.

Experimental

Materials——Pyrrolo[2,3-*d*]pyrimidines (I; **1**—**8**, II; **9**—**11**),[16a] 1,3-dimethyl-6-mercaptouracils (III; **12**—**15**),[16a] and thieno[2,3-*d*]pyrimidines (IV; **16**—**19**)[16a] were the gifts of Professor H. Ogura of Kitasato University. Indole derivatives (V; **20**—**32**, VI; **35**—**42**),[16b-d] benzo[*b*]thiophenes (VII; **43**—**47**),[16e] and 3-cyano-2-methylthio-4-oxo-6,7-dihydrobenzo[*a*]quinolizine (**48**)[16f] were the gifts of Professor G. Kobayashi of University of Nagasaki. Indole derivatives (V; **33**, **34**) were the gifts of Dr. Y. Nitta of this company.

Platelet Aggregation——Biological methods and materials have been partly described in the previous papers.[1,17]

Platelet-rich citrated plasma obtained from male rabbits was used without buffer (a), or with an equal volume (b) or half a volume (c) of isotonic barbital buffer (pH 7.3),[18] by the degree of the aggregation of the platelet-rich citrated plasma. The platelet-rich citrated plasma was stored at 25° for use within 5 hr. The rate and extent of platelet aggregation were measured by the optical density method of Born and Cross[19] by use of Evans EEL 169 aggregation meter. Thus, a cuvette containing 1.0 ml of platelet-rich citrated plasma (PRCP) (a, b or c) preincubated at 37° for 3—5 min was placed in an aggregation meter set at 37°, and allowed to incubate with a test sample dissolved in dimethylsulfoxide or saline with stirring for 3 min. At this point, the platelet-rich citrated plasma was challenged with 10 μl of a solution of adenosine 5′-diphosphate, or 100 μl of a solution of collagen in saline. The final concentration of adenosine 5′-diphosphate and collagen required for aggregation were 10^{-5}M and 0.4 mg/ml respectively. Most of the test samples were dissolved in 10 μl of dimethylsulfoxide[1,6] and compound (**34**) was dissolved in 10 μl of saline, and the final concentrations of each of the test samples were 10^{-4}M. Percent inhibition of aggregation by a test sample was calculated by dividing the maximum deflection of the optical density curve by that observed in the absence of a test sample, then multiplying by 100. Percent inhibitions were not absolute as the sensitivity of platelets to aggregating agents varies from preparation to preparation, and a reference standard, adenosine (10^{-4}M), was tested in every platelet-rich citrated plasma preparation for comparison of the inhibition. Relative potency (Rad) of inhibition of every compound to adenosine compared at 10^{-4}M in the same preparation of platelet-rich citrated plasma was a direct measure of the potency of inhibition. Adenosine showed 30—100% inhibition against adenosine 5′-diphosphate- and collagen-induced platelet aggregation.

Result

Compounds (**1**—**48**) including pyrrolo[2,3-*d*]pyrimidines (**1**—**11**), 1,3-dimethyl-6-mercaptouracils (**12**—**15**), thieno[2,3-*d*]pyrimidines (**16**—**19**), indole derivatives (**20**—**42**), benzo[*b*]thiophenes (**43**—**47**), and benzo[*a*]quinolizine (**48**) were tested *in vitro* as inhibitors of rabbit platelet aggregation induced by adenosine 5′-diphosphate and collagen. The rate and extent of platelet aggregation were measured by the optical density method of Born and Cross.[19] The results are summarized in the Table.

Pyrrolo[2,3-*d*]pyrimidine derivatives (**1**—**11**), 1,3-dimethyl-6-mercaptouracil derivatives (**12**—**15**), and thieno[2,3-*d*]pyrimidine derivatives (**16**—**19**) did not show remarkable inhibitory effects against adenosine 5′-diphosphate- and collagen- induced platelet aggregation. Re-

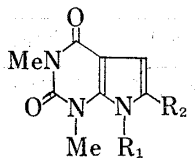
16) *a*) H. Ogura, M. Sakaguchi, and K. Takeda, *Chem. Pharm. Bull.* (Tokyo), **20**, 404 (1972); *b*) G. Kobayashi, Y. Matsuda, R. Natsuki, and Y. Tominaga, *Yakugaku Zasshi*, **90**, 1251 (1970); *c*) G. Kobayashi, Y. Matsuda, R. Natsuki, and Y. Tominaga, *Yakugaku Zasshi*, **91**, 203 (1971); *d*) G. Kobayashi, S. Furukawa, and Y. Matsuda, *Yakugaku Zasshi*, **86**, 1156 (1966); *e*) S. Furukawa and M. Watanabe, *Yakugaku Zasshi*, **92**, 417 (1972); *f*) G. Kobayashi, Y. Matsuda, R. Natsuki, and M. Sone, *Chem. Pharm. Bull.* (Tokyo), **20**, 657 (1972).

17) *a*) K. Kikugawa, K. Iizuka, Y. Higuchi, H. Hirayama, and M. Ichino, *J. Med. Chem.*, **15**, 387 (1972); *b*) K. Iizuka and K. Kikugawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 614 (1972); *c*) K. Kikugawa and K. Iizuka, *J. Pharm. Sci.*, **61**, 1904 (1972).

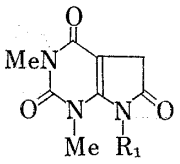
18) K. Kikugawa, K. Iizuka, and M. Ichino, *Chem. Pharm. Bull.* (Tokyo), **20**, 1569 (1972).

19) G.V.R. Born and M.J. Cross, *J. Physiol.*, **168**, 178 (1963).

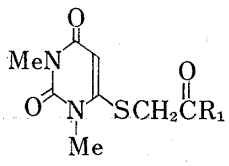
TABLE I. Inhibition of Platelet Aggregation by Heterocyclic Compounds



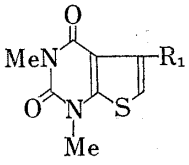
I (1–8)



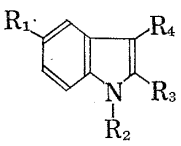
II (9–11)



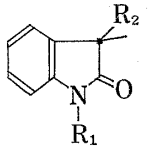
III (12–15)



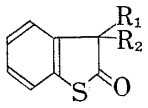
IV (16–19)



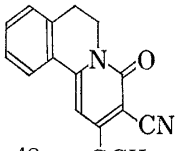
V (20–34)



VI (35–42)

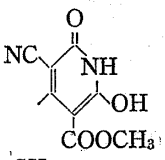
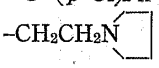
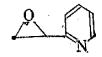
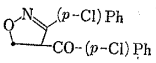
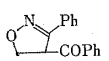
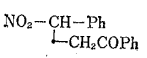
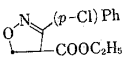
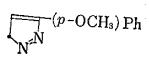
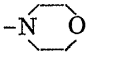


VII (43–47)



48

Compound		R_1	R_2	R_3	R_4	Inhibition of platelet aggregation: ADP-induced PRCP[a]	ADP-induced %[b]	ADP-induced Rad[c]	Collagen-induced PRCP[a]	Collagen-induced %[b]	Collagen-induced Rad[c]
I,	**1**	H	Me			a	25	0.3	c	0	0
	2	H	Ph			a	23	0.3	c	13	0.3
	3	H	*p*-BrPh			a	28	0.3	c	0	0
	4	Me	Me			a	20	0.3	c	0	0
	5	Me	Ph			a	18	0.2	c	3	0.1
	6	Me	*p*-BrPh			a	14	0.2	c	3	0.1
	7	Ph	Ph			a	0	0	a	7	0.1
	8	Ph	*p*-BrPh			a	0	0	a	0	0
II,	**9**	H				a	27	0.3	c	0	0
	10	Me				a	27	0.3	c	0	0
	11	Ph				a	0	0	a	0	0
III,	**12**	Me				a	0	0	a	5	0.1
	13	Ph				a	0	0	a	0	0
	14	*p*-BrPh				a	0	0	a	4	0.1
	15	OEt				a	24	0.4	a	15	0.4
IV,	**16**	Me				a	9	0.1	a	11	0.3
	17	Ph				a	6	0.1	a	19	0.4
	18	*p*-BrPh				a	0	0	c	4	0.1
	19	OH				a	20	0.4	c	11	0.3
V,	**20**	H	H	Ph	$-C(=S)-NHCH_2Ph$	a	0	0	a	0	0
	21	H	H	$-CON(CH_3)_2$	$-C(=S)-SCH_3$	a	12	0.1	a	7	0.2
	22	H	Me	Me	$-C(=S)-N\langle\rangle O$	a	0	0	b	67	0.7
	23	H	Me	Me	$-C(=S)-NHCH_2CH_2-OH$	a	6	0.1	b	79	0.9
	24	H	Me	Me	$-C(=S)-NHCH_2Ph$	a	30	0.3	a	0	0
	25	H	$-C(=S)-SCH_3$	H	$-C(CN)=C(SCH_3)_2$	a	21	0.2	b	53	0.9
	26	H	$-C(=S)-NHCH_2Ph$	H	$-C(CN)=C(SCH_3)_2$	a	11	0.1	b	77	0.8
	27	H	$-C(=S)-N\langle\rangle O$	H	$-C(CN)=C(SCH_3)_2$	a	61	0.6	b	54	0.6

Compound	R_1	R_2	R_3	R_4	Inhibition of platelet aggregation: ADP-induced PRCP[a]	% [b]	Rad [c]	Collagen-induced PRCP[a]	% [b]	Red [c]
28	H	$-\overset{O}{\overset{\|}{C}}-CH_3$	$-\overset{SCH_3}{\overset{\|}{C}}=C\langle^{COOCH_3}_{CN}$	OH	a	25	0.3	a	10	0.2
29	H	$-\overset{S}{\overset{\|}{C}}-NHN=C\langle^{CH_3}_{CH_3}$	H	$-\overset{CN}{\overset{\|}{C}}=C(SCH_3)_2$	a	41	0.5	a	32	0.6
30	H	H	H		a	13	0.1	a	2	0.1
31	H	H	$-CON\langle^{CH_3}_{CH_3}$	$-\overset{CH_3}{\overset{\|}{C}}=C\langle^{CN}_{CN}$	a	28	0.3	a	9	0.3
32	H	$-\overset{O}{\overset{\|}{C}}-CH_3$	$-\overset{SCH_3}{\overset{\|}{C}}=NSO_2-(p\text{-}CH_3)Ph$	OH	a	24	0.3	a	19	0.7
33	H	$-\overset{O}{\overset{\|}{C}}-(p\text{-}Cl)Ph$	Ph	H	b	13	0.1	c	100	1.0
34	MeO		Ph	H	b	49	1.1	c	100	2.0
VI, 35	H				a	3	0	a	0	0
36	Me				a	36	0.4	a	28	0.3
37	Me				a	30	0.3	a	17	0.2
38	Me				a	36	0.4	a	43	0.5
39	Me				a	37	0.4	a	53	0.6
40	Me				a	22	0.4	a	6	0.1
41	Me				a	14	0.3	a	17	0.5
42	Me				a	5	0.1	a	0	0
VII, 43	OH	$-NH-(p\text{-}CH_3)Ph$			a	67	0.7	a	50	0.6
44	OH	$-NHCH_2CH_2OH$			a	11	0.5	a	0	0
45	OH				a	93	1.0	a	34	0.4
46	$=CH-\overset{O}{\overset{\|}{C}}-Ph$				a	88	1.0	a	54	1.0
47	$=CH-\overset{O}{\overset{\|}{C}}-CH_3$				a	100	1.0	a	100	1.2
48					a	61	0.8	a	36	1.0

a) Platelet-rich citrated plasma without buffer (a), or with an equal volume (b) or half a volume (c) of buffer.
b) % inhibition was not absolute.
c) Relative potency (Rad) of every compound to adenosine was a direct measure of potency. Ph=phenyl

latively potency (Rad) of inhibition of these compounds to adenosine compared at 10^{-4}M did not exceed 0.5.

Among the indole derivatives (**20**—**42**) several compounds were found active as platelet aggregation inhibitors. Compounds (**22, 23, 25, 26, 32, 33**) were as effective as adenosine against collagen-induced platelet aggregation. Compounds (**27, 29, 34**) were active against both adenosine 5′-diphosphate- and collagen-indiuced platelet aggregation. Of these indole derivatives, N(1-pyrrolidinyl)ethyl-2-phenyl-5-methoxy-indole (**34**) was found most effective, and it showed rather stronger inhibition than adenosine at 10^{-4}M against adenosine 5′-diphosphate- and collagen-induced platelet aggregation. This series of compounds are structural analogs of a nonsteroidal antiinflammatory drug such as indomethacin which have been reported to inhibit collagen-induced platelet aggregation.[20]

Benzo[*b*]thiophene derivatives (**43**—**47**) had rather strong inhibitory activity except compound (**44**) against both adenosine 5′-diphosphate- and collagen-induced platelet aggregation. 3-Cyano-2-methylthio-4-oxo-6,7-dihydrobenzo[*a*]quinolizine (**48**) was also effective as an inhibitor of platelet aggregation mediated by both inducers. These compounds (**43, 45, 46, 47, 48**) could be appreciated to be of new classes of platelet aggregation inhibitors and these are valuable compounds for further pharmacological studies.

In summary, several heterocyclic compounds were found effective *in vitro* as inhibitors of platelet aggregation. It is of interest that some of them inhibited both adenosine 5′-diphosphate- and collagen-induced platelet aggregation and others inhibited only the aggregation mediated by collagen. The utility of these active compounds as antithrombotic agents depends on further pharmacological investigations.

Acknowledgement The authors wish to express their sincere thanks to Professor H. Ogura of Kitasato University, Professor G. Kobayashi of University of Nagasaki, and Dr. Y. Nitta, the Director of this company, for their kindness in supplying the compounds. Thanks are also due to Mr. T. Nakamura, the Manager of the Laboratory and to Mrs R. Toyoshima for his encouragements and for her technical assistances respectively.

20) J.R. O'Brien, *Lancet*, **1968**, 894.

[Chem. Pharm. Bull. 21(5)1155—1157(1973)]

UDC 547.724.1′458.02. : 581.192

Isolation of 5-Hydroxymethylfurfural from *Trachelospermum asiaticum* var. *intermedium*

Sansei Nishibe, Sueo Hisada, and Isao Inagaki

Faculty of Pharmaceutical Sciences, Nagoya City University[1]

(Received November 20, 1972)

As one of carbohydrate components of several *Trachelospermum* species (Apocynaceae), we have reported the isolation of dambonitol (1,3-di-O-methyl-*myo*-inositol).[2]

This report is concerned with the isolation and identification of sucrose and 5-hydroxymethylfurfural from the stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai.

The procedure of extraction was as described in the experimental section. The extract with chloroform–methanol (2:1) was subjected to a column chromatography of activated charcoal and eluted successively with water, methanol–water (1:99), methanol–water (1:1) and methanol alone. The colorless grains (I), $C_{12}H_{22}O_{11}$, mp 176—179°, $[\alpha]_D^{21}$ +66.0° (water)

1) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
2) S. Nishibe, S. Hisada, and I. Inagaki, *Yakugaku Zasshi*, **93**, 539 (1973).