Chem. Pharm. Bull. 15(8)1101~1106(1967)

UDC 615.771.7 : 547.462.3.09

136. Hidetaka Yuki,*2 Tadahiro Yamamoto, Yasuo Tohira, Bunya Aoki, Tokio Kano, and Tsuyoshi Yamazaki: Studies on Antiviral Agents. II.*3 Synthesis and Biological Activity of Maleimide Derivatives.

(*Research Laboratories, Chugai Pharmaceutical Co., Ltd.**1)

N- and 2-substituted maleimides were synthesized as antiviral agents. Though all were negative against Adenovirus type 5 and Newcastle disease virus, some compounds were found to be active against certain bacteria and fungi.

(Received March 15, 1966)

It has been generally known that sulfhydryl reagents act on the various enzymes which play an important role in biological systems, and consequently inhibit growth of a number of bacteria and fungi. In the part I of this series, it has been reported that some sulfhydryl reagents diminished the replication of Adenovirus type 5. Among the compounds tested, N-phenylmaleimide and diphenyl maleate reduced the infectivity of the virus while other maleic acid derivatives failed to do so regardless of high reaction rate of certain compounds, *e.g.* N-ethylmaleimide, with the SH-group of methyl N-acetyl cysteinate. Friedman, *et al.*[1] reported that an introduction of a methyl group to the position 2 of maleimide decreased the reactivity toward the sulfhydryl group. Although the literatures[2,3] contain several references to the synthesis of 2-arylmaleimides, the investigation has not been referred to the biological examination of these compounds. In the current work, an aryl group was introduced to the position 2 of N-substituted maleimides in the hope of developing any characteristic activity of the maleimide series. The aryl group introduced is expected to exert if any steric and/or electronic effect on the chemical activity, consequently biological activity of the maleimides depending on the position and nature of the substituent in the benzene ring.

Maleamic acids (I) were first synthesized from maleic anhydride and corresponding amines by the method of Liwschitz *et al.*[4] Several methods have been presented to cyclize the maleamic acid to maleimide : simple fusion,[5] heating in medicinal paraffin,[6] treatment with dehydrating agents such as acetic anhydride,[7,8] phosphorus pentoxide[9,10] or polyphosphoric acid.[11] In the present investigation, acetic anhydride method was applied for the preparation of N-substituted maleimides (II). Namely, N-arylmaleimides

*1 Takadaminami-cho, Toshimaku, Tokyo (由岐英剛, 山本忠宏, 東平靖雄, 青木文彌, 加納外記雄, 山崎強志).

*2 Present address : Faculty of Pharmaceutical Sciences, Osaka University, Toneyama, Toyonaka-city, Osaka.

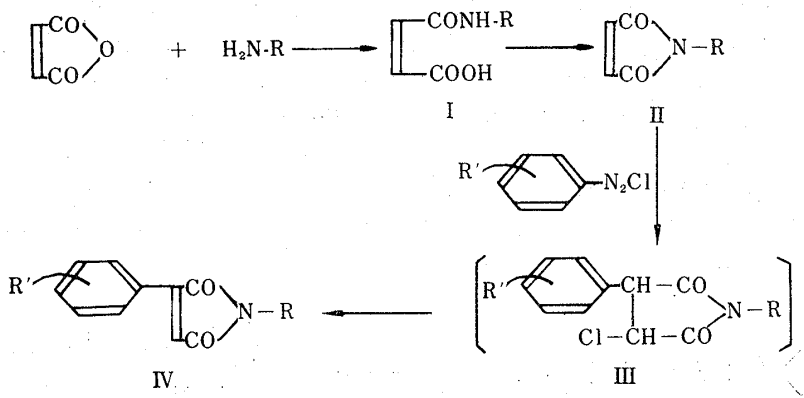
*3 Part I : This Bulletin, **14**, 139 (1966).

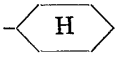
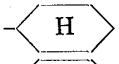
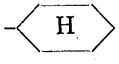
1) E. Friedman, *et al.* : Brit. J. Pharmacol., **4**, 104 (1949).
2) C. S. Rondestvedt, Jr., *et al.* : J. Am. Chem. Soc., **77**, 2313 (1955).
3) *Idem* : *Ibid.*, **78**, 6115 (1956).
4) Y. Liwschitz, *et al.* : *Ibid.*, **78**, 3069 (1956).
5) A. Pirtti, *et al.* : Gazz. chim. ital., **261**, 439 (1896).
6) D. H. Marrian : J. Chem. Soc., **1949**, 1515.
7) C. K. Sauers, *et al.* : U. S. Pat., 3,018,290, Jan. 23 (1962).
8) N. E. Searles : U. S. Pat., 2,444,536, July 6 (1948).
9) M. Z. Barakat, *et al.* : J. Chem. Soc., 4133, (1957).
10) W. R. Roderick : J. Am. Chem. Soc., **79**, 1710 (1957).
11) Y. Kanaoka, *et al.* : This Bulletin, **12**, 127 (1964).

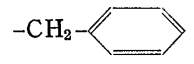
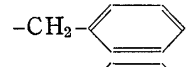
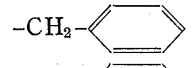
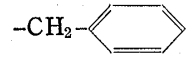
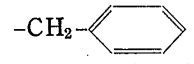
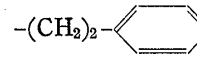
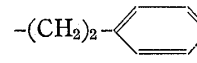
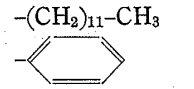
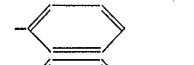
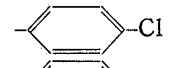
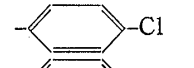
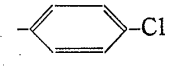
Table I. N, 2-Disubstituted Maleimides

Compounds	m.p. (°C)	Recrystn.	Analysis (%) Calcd. C	H	N	Found C	H	N
Ⅳ 1	125.5～126	MeOH	71.62	5.51	6.96	71.21	5.57	6.84
2	121.5	C_6H_6	72.54	6.09	6.51	72.61	5.81	6.46
3	117～118	EtOH	61.18	4.28	5.94	60.80	4.36	5.78
4	146.0	C_6H_6	51.45	3.60	5.00	51.70	3.28	4.71
5	239.41	EtOH	73.34	6.59	6.11	73.10	6.55	5.79
6	88～89	EtOH	62.64	6.02	5.62[a)]	62.67	5.84	5.41
7	87～88	EtOH	58.53	4.09	11.38	58.41	4.17	11.42
8	166～167	C_6H_6	56.52	4.38	10.14	56.49	4.58	10.21
9	91～91.5	MeOH	72.54	6.09	6.51	72.09	6.10	6.51
10	105～106	MeOH	73.34	6.59	6.11	72.63	6.85	5.99
11	119～120	EtOH	61.15	4.28	5.94	61.43	4.31	5.97
12	109～110	EtOH	53.36	3.36	5.19	53.55	3.49	5.18
13	115～116	EtOH	62.53	4.84	5.61	62.37	4.74	5.68
14	126～127	C_6H_6–EtOH	51.35	3.23	9.98	51.06	3.21	10.36
15	128	EtOH	58.77	4.55	5.27	58.58	4.64	5.42
16	148～148.5	MeOH	78.27	6.71	5.49	75.32	6.95	5.45
17	139～142	EtOH	57.53	4.79	4.19	57.22	4.90	4.16
18	189～190	EtOH	63.79	5.37	9.33	64.11	5.17	9.22
19	142	EtOH	59.21	4.66	4.32	58.93	4.51	4.41
20	193～196	EtOH–C_6H_6	61.85	5.45	8.48	62.16	5.84	8.42
21	147.5～148	EtOH	77.55	4.98	5.32	77.50	5.19	5.29
22	125～122	EtOH	77.96	5.45	5.05	77.87	5.43	4.94
23	153～154	EtOH	69.25	4.08	4.74	69.23	4.22	4.69
24	148～150	EtOH	59.70	3.85	4.07	59.63	3.53	4.07
25	130～131.5	EtOH	66.23	3.92	9.09	66.19	3.80	9.04
26	135～137	EtOH	60.71	3.93	3.93	60.45	3.98	3.80
27	122～124	EtOH	67.07	4.38	8.69	66.83	4.46	9.05
28	70～71	MeOH	74.36	8.95	3.77	73.99	8.87	3.77
29	136	EtOH–AcOEt	77.55	4.98	5.32	77.38	5.25	5.09
30	178.0	C_6H_6	60.40	2.85	4.40	60.51	2.80	4.46
31	150.5	EtOH–C_6H_6	67.74	3.55	4.94	68.10	3.49	4.37
32	173.5	EtOH–AcOEt	68.58	4.06	4.71	68.59	4.05	4.47
33	215.5	C_6H_6	60.40	2.85	4.40	60.69	2.96	4.11
34	191.0	EtOH–AcOEt	58.46	2.76	8.54	58.67	2.96	8.50
35	183.0	EtOH–C_6H_6	65.08	3.86	4.47	65.75	3.97	4.09
36	191.0	AcOEt	66.23	3.93	9.09	66.51	4.12	9.25
37	212.0	AcOEt	58.46	2.76	8.52	58.68	2.99	8.57
38	118.5	MeOH	77.55	4.98	5.32	77.87	4.88	5.71
39	161.5	EtOH–C_6H_6	77.96	5.45	5.05	77.88	5.54	4.98
40	171.5	C_6H_6	68.58	4.06	4.71	68.27	4.05	4.49
41	142.5	MeOH	66.28	3.93	9.09	66.82	4.13	9.11
42	132.0	EtOH–C_6H_6	73.71	5.16	4.78	73.46	5.30	4.66
43	175.5	AcOEt	74.25	5.58	4.56	74.17	5.47	4.37
44	183.0	EtOH–AcOEt	65.96	4.31	4.27	65.99	4.35	4.09
45	140.0	MeOH	70.58	5.30	4.33	70.42	5.06	4.33

a) Calculated as one molar of water of crystallization.



	R	R′
Ⅳ 1	Et	H
2	Et	*p*-CH_3
3	Et	*p*-Cl
4	Et	*p*-Br
5	Et	*p*-Et
6	Et	*o*-CH_3O
7	Et	*m*-NO_2
8	Et	2-MeO, 4-NO_2
9	iso-C_3H_7	H
10	iso-C_3H_7	*p*-CH_3
11	$(CH_2)_2Cl$	H
12	$(CH_2)_2Cl$	*p*-Cl
13	$(CH_2)_2Cl$	*p*-CH_3
14	$(CH_2)_2Cl$	*p*-NO_2
15	$(CH_2)_2Cl$	*p*-CH_3O
16		H
17		*p*-Br
18		*p*-NO_2
19		3,4-diCl
20		2-MeO, 4-NO_2

	R	R′
Ⅳ21		H
22		*p*-CH_3
23		*p*-Cl
24		*p*-Br
25		*o*-NO_2
26		*p*-Br
27		*p*-NO_2
28		*o*-CH_3O
29		*p*-CH_3
30		3,4-diCl
31		H
32		*p*-CH_3
33		*p*-Cl

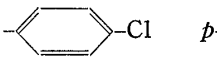
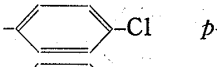
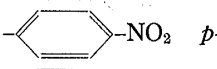
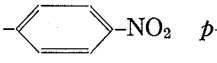
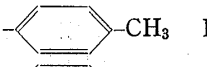
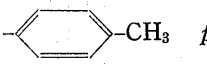
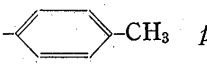
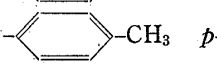
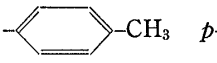
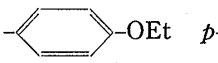
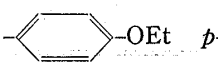
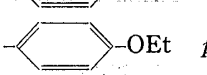
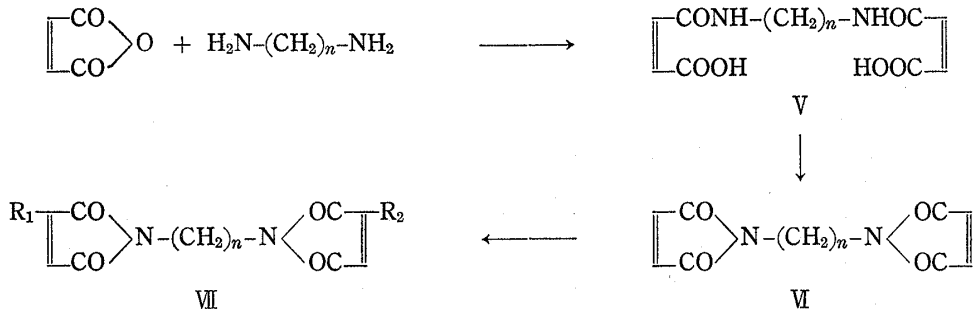
	R	R′
Ⅳ34		*p*-NO_2
35		*p*-CH_3O
36		*p*-CH_3
37		*p*-Cl
38		H
39		*p*-CH_3
40		*p*-Cl
41		*p*-NO_2
42		*p*-CH_3O
43		*p*-CH_3
44		*p*-Cl
45		*p*-CH_3O

Chart 1.

were prepared by heating the maleamic acid with a mixture of acetic anhydride and sodium acetate, and N-alkylmaleimides were prepared by refluxing a mixture of the maleamic acid, triethylamine, acetic anhydride, and benzene. To introduce an aryl group to the position 2 of the maleimides, the aryl diazonium chloride was reacted with maleimide at pH 3~4 to yield 2-aryl-3-chlorosuccinimide (Ⅲ), which was converted to 2-arylmaleimide (Ⅳ) without isolation by treatment with 2,4-lutidine as a dehydrochloridating agent. Introduction of *p*-substituted phenyl proup could be performed with comparative ease, while introduction of *o*- and *m*-substituted phenyl group failed in many cases. The compounds synthesized were shown in the Chart 1 and the Table I.

As a maleimide is one of the alkylating agents, preparation of bifunctional maleimides is of interest from the biological point of view. To prepare such compounds, tri-, and hexamethylenediamine were treated with maleic anhydride and were brought to N,N′-polymethylenbis (2,2′-diaryl)maleimides (Ⅶ) by the methods described above as is shown in the Chart 2. In some cases, reaction with two moles of diazonium compound yielded only 2-monoaryl compound (Ⅶ, R_2=H) without producing any

2,2′-diaryl maleimide derivative. In these cases, however, reaction with four moles of diazonium compounds yielded the desired 2,2′-diaryl maleimide derivatives. As another type of bifunctional alkylating agents, synthesis of N-β-chloroethyl-2-maleimides (Ⅳ, R=-CH_2CH_2Cl) was undertaken. β-Chloroethylamine was reacted with maleic anhydride to yield N-β-chloroethylmaleamic acid (I,R=-CH_2CH_2Cl), which was cyclized with acetic anhydride and sodium acetate to N-β-chloroethylmaleimide (Ⅱ,R=CH_2CH_2Cl) exceptionally using the method for preparation of N-aryl maleimide. 2-Aryl groups were introduced as usual.



Ⅶ

	n	R_1	R_2
Ⅶ 1	3	o-Cl-C_6H_4-	o-Cl-C_6H_4-
2	3	m-Cl-C_6H_4-	m-Cl-C_6H_4-
3	3	p-Cl-C_6H_4-	p-Cl-C_6H_4-
4	3	p-CH_3-C_6H_4-	p-CH_3-C_6H_4-
5	3	p-NO_2-C_6H_4-	p-NO_2-C_6H_4-
6	3	p-CH_3O-C_6H_4-	p-CH_3O-C_6H_4-
7	3	3,4-diMe-C_6H_3-	3,4-diMe-C_6H_3-
8	3	3,4-diCl-C_6H_3-	3,4-diCl-C_6H_3-
9	3	m-Cl-C_6H_4-	H
Ⅶ 10	3	m-CH_3-C_6H_4-	H
11	6	o-Cl-C_6H_4-	o-Cl-C_6H_4-
12	6	m-Cl-C_6H_4-	m-Cl-C_6H_4-
13	6	p-CH_3-C_6H_4-	p-CH_3-C_6H_4-
14	6	p-CH_3O-C_6H_4-	p-CH_3O-C_6H_4-
15	6	3,4-diMe-C_6H_3-	3,4-diMe-C_6H_3-
16	6	m-Cl-C_6H_4-	H
17	6	p-Cl-C_6H_4-	H

Chart 2.

Table Ⅱ. N,N′-Tri-and Hexamethylene Bis-maleimides

Compounds	m.p. (°C)	Recrystn.	Analysis (%) Calcd. C	H	N	Found C	H	N
Ⅶ 1	146~148	EtOH	60.68	3.54	6.15	60.79	3.66	6.24
2	176~179	acetone	60.68	3.54	6.15	60.99	3.79	6.27
3	189.5~190	benzene-THF[a]	60.68	3.54	6.15	61.12	3.62	6.24
4	202~203	THF	72.45	5.35	6.76	72.59	5.46	6.75
5	159~160	EtOH-benzene	57.99	3.38	11.74	57.84	3.92	11.86
6	226~228	dioxane	67.26	4.97	6.28	66.97	5.02	6.28
7	162~163	benzene	73.62	5.49	6.36	73.39	5.90	6.43
8	203.5~204.5	dioxane	52.70	2.69	5.34	52.90	2.77	5.31
9	120~125	MeOH	59.23	3.77	8.13	58.88	4.27	8.45
10	108~111	EtOH-H_2O	66.69	4.97	8.64	66.30	4.96	8.56
11	136.5~139	EtOH-C_6H_6	62.79	4.46	5.63	62.64	4.20	5.50
12	152~154	EtOH-C_6H_6	62.79	4.46	5.63	62.52	4.58	5.58
13	186.5~187.5	benzene-THF	73.67	6.18	6.14	73.67	6.26	6.13
14	216~217	dioxane	68.84	5.78	5.73	68.49	5.87	5.72
15	151~152	benzene	74.36	6.66	5.78	74.42	6.48	5.81
16	105~107	EtOH-benzene	62.26	4.70	7.26	62.28	5.03	7.22
17	128~130	EtOH-benzene	62.26	4.70	7.26	62.00	4.94	7.24

a) THF : tetrahydrofuran

Antiviral activity of the maleimide compounds synthesized was all negative against Newcastle disease virus and Adenovirus type 5. However, some compounds were found to be active against certain bacteria and fungi. These were listed in the Table Ⅲ, and the compounds not listed in the table were all negative. Anti-tumor activity is now being tested at Cancer Chemotherapy National Service Center, N.I.H.

Table Ⅲ. Biological Activities of Maleimides (minimum inhibitory concentration, μg./ml.)

Strains / Compounds	*Staph.*[a]	*T.*[b]	*C.*[c]	*Shig.*[d]	*Tric.*[e]	*Pi.*[f]	*Xant.*[g]
Ⅳ 2	5.0	5～10	1.25～2.5	20	—	1.57	1.56
3	1.25～2.5	1.25	1.25	5	10～20	1.0	100
4	2.5	1.25	1.25	5	—	0.78	3.13
6	2.5	2.5～5	5	20	20	—	—
7	10	5～10	5	20	10～20	5	10
8	2.5	2.5～5	2.5～5	5	5	—	—
10	5	2.5	5	5	5	5～10	5～10
11	—	2.5	2.5～5	—	—	—	—
12	—	2.5～5	1.25～2.5	—	—	—	—
13	—	2.5	2.5～5	—	—	—	—
15	—	2.5～5	20	—	—	—	—
18	5	5	5	5	2.5～5	100	100
21	2.5	5	5	2.5	5	10	100
23	1.25～2.5	5～5	5	10	5	100	100
24	2.5	5	5	2.5	5	100	100
25	5	2.5	5	5	5	100	5
Ⅶ 1	10	10～20	20	20	—	100	5

a) *Staph. Aureus* 209 p
b) *T. Mentagrophytes* T-1
c) *C. albicans* 2
d) *Shig. flexneri* 2a
e) *Trichom. vaginalis* J
f) *Piricularia oryzae*
g) *Xanthomonas oryzae*

Experimental

General Method

N-Maleamic Acid——The amine dissolved in anhyd. ether or benzene was added to an ice-cold solution of equimolar maleic anhydride in anhyd. ether or benzene with stirring. N-Maleamic acid precipitated was collected and recrystallized. Yield, 80～100%.

N-Aryl Maleimide——A mixture of N-arylmaleamic acid (1.0 mole), Ac_2O (6 moles) and AcONa (0.1 mole) was heated at 90° for 30～60 mins. with stirring. The mixture was then poured into ice water, and the precipitate was filtered, washed with water, and recrystallized from MeOH. Yield, 50～70%.

N-Alkylmaleimide——A suspension of N-alkyl maleamic acid (1.0 mole), in a mixture of Et_3N (2.0 moles) and anhyd. benzene was brought lo reflux, and Ac_2O (1.0 mole) was added to the mixture dropwise for 1 hr. After cooling, the benzene solution was washed with 10% NaOH to neutralize the AcOH produced, and with satd. NaCl soln., and was dried. The solution was evaporated to dryness, and the residue was recrystallized from a small volume of benzene or EtOH. Yield, 30～60%.

Dimaleamic Acid——One mole of alkylenediamine in 90% acetic acid was added to two moles of maleic anhydride dissolved in AcOH. After several mins., crystalline dimaleamic acid separated was filtered, washed with benzene, and recrystallized. Yield, 80～90%.

Dimaleimide——A mixture of dimaleamic acid (1.0 mole), Et_3N (4.0 moles) and Ac_2O (2.0 moles) was heated to 90° for 10 mins., and allowed to stand at room temperature for 3 hr. Precipitate was filtered, washed with benzene, and recrystallized. If not precipitated, the mixture was poured into an ice-water, and the precipitate was filtered, washed with water and recrystallized. Yield, 30～50%.

Arylation of Maleimides——The aryl amine dissolved in three equivalents of dil. HCl was diazotized with 30% $NaNO_2$ solution with vigorous stirring at 0～5°. This diazonium solution was adjusted to pH 3～4 with satd. AcONa solution, and was poured into an ice-cold solution of 1.0～1.5 moles of the maleimide dissolved in acetone. To this solution was added 0.15 mole of cuprous chloride. The mixture was stirred at 0～5° for 30 mins., and allowed to stand at room temperature for 3 hr. The acetone was

evaporated at a reduced pressure without heating. The residue was extracted with benzene. Two to three moles of 2.6 lutidine was added to this benzene solution and the mixture was warmed gently until crystalline lutidine hydrochloride was separated. The benzene was washed with water to dissolve the hydrochloride, and washing with 10% HCl, 10% NaOH, and water was followed. Benzene was evaporated to dryness at a reduced pressure, and the residue was recrystallized. Yield, 15~50%.

The authors wish to express their deep gratitude to Dr. T. Akiba, the director of the research laboratories, and Drs. Y. Nitta and H. Sano, the heads of the department, for their kind advices and encouragements throughout this investigation. Thanks are also due to the members in the sections of analysis and microbiology for their elemental analysis and biological assay.