[Chem. Pharm. Bull. 31(4)1277—1282(1983)]

1(2*H*)-Isoquinolones as Potential Antiallergic Agents

MASAYASU KIMURA*,[a] ISAMI WAKI,[a] YOJI DEGUCHI,[a]
KOUJI AMEMIYA[b] and TETSUYA MAEDA[b]

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University,[a] 2630, Sugitani, Toyama 930-01, Japan and Medical Drug Research Laboratories, Kaken Chemical Co., Ltd.,[b] 28-8, Honkomagome 2-chome, Bunkyo-ku, Tokyo 113, Japan

(Received October 7, 1982)

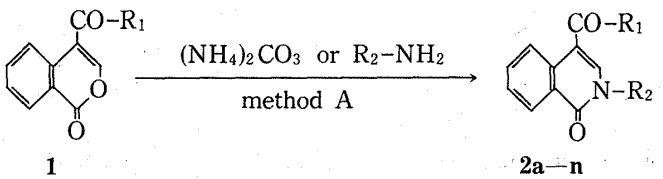
A series of 1(2*H*)-isoquinolones was prepared by reaction of 4-acylisocoumarins with ammonium carbonate or primary amines in acetic acid. These compounds, after a 15-min pretreatment, inhibited histamine release from isolated rat mast cells, and some of them were potent when tested on rat passive cutaneous anaphylaxis (PCA assay 3 h after *i.p.* injection). The activities of these compounds were, however, lower than that of the already known 4-(4-carboxybenzoyl)isocoumarin.

Keywords——1(2*H*)-isoquinolone; 4-acylisocoumarin; antiallergic; histamine release inhibition; PCA test

The potent antiallergic agent KIT-302 [4-(4-carboxybenzoyl)isocoumarin][1] has emerged from experiments to synthesize analogues of oosponol,[2] a metabolic product isolated from *Oospora astringens* (obtained from house dust from the home of an asthmatic patient). KIT-302 possesses potent inhibitory activity on histamine release from isolated rat mast cells, but lower concentrations of KIT-302 tend to potentiate the release.[3] Isocoumarins react with primary amines to yield 1(2*H*)-isoquinolones.[4] Thus, when KIT-302 is incubated with mast cells, it might bind to the amino groups of a key enzyme in the histamine release pathway, and its modification might lead to the potentiation. With this idea in mind, we felt that it might be fruitful to synthesize 1(2*H*)-isoquinolone analogues. We also attempted to reduce the potentiating activity of KIT-302 by preparing the 3-methyl-substituted compound. In addition, synthesis of 6,7-methylenedioxy compounds was performed in the hope of obtaining improved potency.[5]

Chemistry

A series of 1(2*H*)-isoquinolones (**2**) was prepared from isocoumarins (**1**) according to the method of Kanevskaya.[4] The reaction of isocoumarins[6] (**1**) with ammonium carbonate or primary amines in acetic acid afforded the products (**2**) (Chart 1).



1 **2a—n**

2a : $R_1=OH$, $R_2=H$
2b : $R_1=CH_3$, $R_2=H$
2c : $R_1=CH_2OCOCH_3$, $R_2=H$
2d : $R_1=CH_3$, $R_2=CH_3$
2e : $R_1=CH_3$, $R_2=CH_2CH_3$
2f : $R_1=CH_3$, $R_2=(CH_2)_3CH_3$
2g : $R_1=CH_3$, $R_2=CH_2C_6H_5$
2h : $R_1=CH_3$, $R_2=C_6H_5$
2i : $R_1=CH_3$, $R_2=CH_2CO_2H$
2j : $R_1=CH_3$, $R_2=N(CH_2)_3N(CH_3)_2$
2k : $R_1=C_6H_4$-4-CO_2H, $R_2=H$
2m: $R_1=C_6H_4$-4-CO_2H, $R_2=CH_3$
2n : $R_1=C_6H_4$-4-CO_2H, $R_2=(CH_2)_3CH_3$

Chart 1

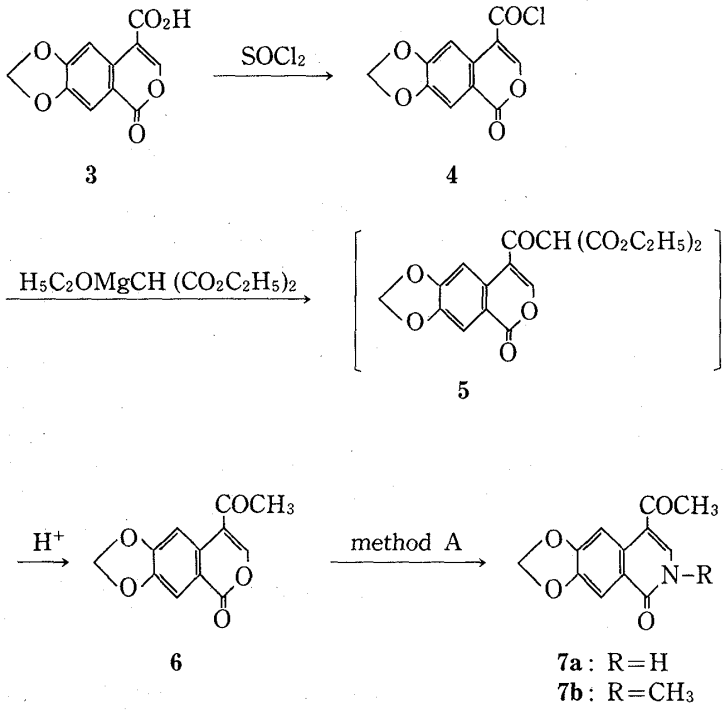


Chart 2

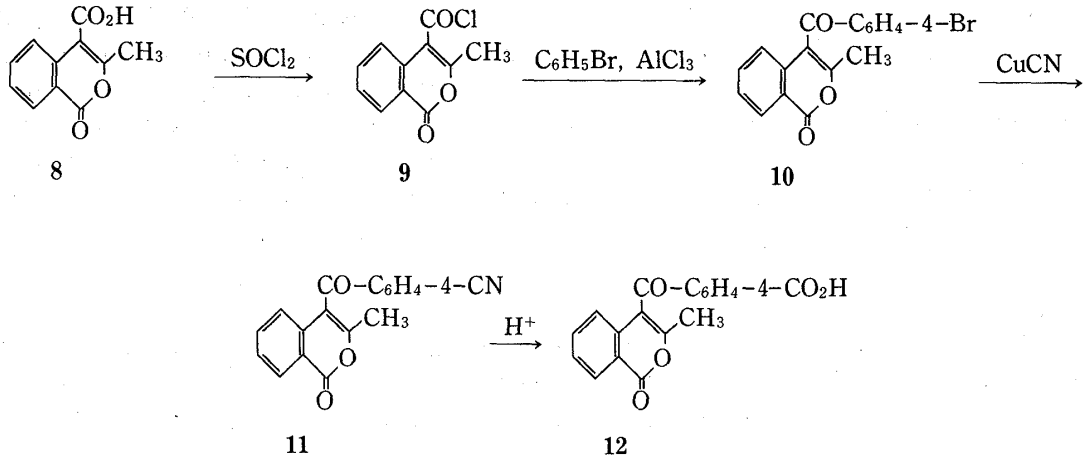
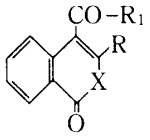


Chart 3

Treatment of 4-chloroformyl-6,7-methylenedioxyisocoumarin (**4**), synthesized from **3**,[7] with ethoxymagnesiummalonate in chlorobenzene gave the intermediate (**5**), which was then hydrolyzed to 4-acetyl-6,7-methylenedioxyisocoumarin (**6**). 4-Acetyl-6,7-methylenedioxy-1(2*H*)-isoquinolones (**7**) were prepared from **6** by the same method as used for **2** (Chart 2).

The isocoumarin **12** was prepared by the synthetic route shown in Chart 3. By means of the Friedel Crafts reaction, 4-(4-bromobenzoyl)-3-methylisocoumarin (**10**) was prepared from **9**, which was synthesized from 3-methylisocoumarin-4-carboxylic acid[8] (**8**). The heating of **10** with CuCN in *N*,*N*-dimethyl formamide (DMF) furnished 4-(4-cyanobenzoyl)-3-methylisocoumarin (**11**) which was hydrolyzed to 4-(4-carboxybenzoyl)-3-methylisocoumarin (**12**).

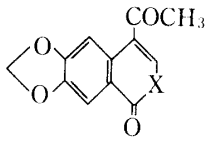
TABLE I. 1(2*H*)-Isoquinolones and Isocoumarins



Compd.	X	R	R_1	mp (°C)	Crystn. solvent	Yield (%)	Formula[a]	HR inhibn. IC_{50}, mM	Rat PCA ID_{50} mmol/kg
2a[b]	NH	H	OH	296—297dec.[c]	DMF		$C_{10}H_7NO_3$	>5	>0.2
2b	NH	H	CH_3	242.5—243.5[d]	$CHCl_3$-EtOH	52.9	$C_{11}H_9NO_2$	>5	0.014
2c	NH	H	CH_2OCOCH_3	245.5(dec.)	MeOH	27.0	$C_{13}H_{11}NO_4$	>4	0.10
2d	NCH_3	H	CH_3	144—145	C_6H_6	45.8	$C_{12}H_{11}NO_2$	1.2(1.0—1.5)	0.084
2e	NCH_2CH_3	H	CH_3	125.5—126.5	$CHCl_3$-hexane	52.8	$C_{13}H_{13}NO_2$	3.0(0.86—10)	0.12
2f	$N(CH_2)_3CH_3$	H	CH_3	43—44.5	C_6H_6-hexane	75.4	$C_{15}H_{17}NO_2$	>4	>0.2
2g	$NCH_2C_6H_5$	H	CH_3	158—159	C_6H_6	87.7	$C_{18}H_{15}NO_2$	>4	0.10
2h	NC_6H_5	H	CH_3	106.5—107.5	C_6H_6-hexane	58.5	$C_{17}H_{13}NO_2$	>4	>0.2
2i	NCH_2CO_2H	H	CH_3	227—228	C_6H_6-MeOH	61.6	$C_{13}H_{11}NO_2$	>4	>0.2
2j	$H(CH_2)_3N(CH_3)_2$	H	CH_3	213—214	Acetone-MeOH	47.1	$C_{16}H_{20}N_2O_2 \cdot HCl \cdot 1/2H_2O$	0.71(0.61—0.82)	>0.1
2k	NH	H	C_6H_4-4-CO_2H	>300	DMF-MeOH	82.9	$C_{17}H_{11}NO_4$[e]	3.2(2.5—4.1)	0.061
2m	NCH_3	H	C_6H_4-4-CO_2H	283—284.5	$CHCl_3$-MeOH	72.3	$C_{18}H_{13}NO_4$	0.69(0.63—0.77)	>0.1
2n	$N(CH_2)_3CH_3$	H	C_6H_4-4-CO_2H	181—182	MeOH	67.3	$C_{21}H_{19}NO_4 \cdot CH_3OH$	0.71(0.62—0.82)	0.059
12	O	CH_3	C_6H_4-4-CO_2H	243—244	EtOH	71.4	$C_{18}H_{12}O_5$	0.33(0.29—0.42)	>0.1
1a[f]	O	H	C_6H_4-4-CO_2H	254—257	EtOH		$C_{17}H_{10}O_5$	0.041 (0.036—0.045)	0.014
	Disodium cromoglycate							0.68 (0.55—0.83)×10^{-3}[g]	0.078

a) Analysis values for C, H and N were within ±0.4% of calculated values except where otherwise noted.
b) Synthesized according to the lit.[9]
c) Lit.[9] mp 297°C.
d) Lit.[10] mp 133—135°C.
e) C: Calcd, 69.62; Found 69.04.
f) Synthesized according to the lit.[5a]
g) A 30-s pretreatment. When incubation for 15 min was used, no significant inhibition was seen.

TABLE II. 6, 7-Methylenedioxy Compounds



Compd.	X	mp (°C)	Crystn. solvent	Yield (%)	Formulaa)	HR inhibn. IC_{50}, mM	Rat PCA ID_{50} mmol/kg
6	O	234—235	$CHCl_3$	52.8	$C_{12}H_8O_5$[b]	>4	>0.2
7a	NH	291.5—292.5 (dec.)	C_6H_6-DMF	91.3	$C_{12}H_9NO_4$	>4	>0.2
7b	NCH_3	221—222	$CHCl_3$	56.2	$C_{13}H_{11}NO_4$	>4	>0.2

a) Analysis values for C, H and N were within ± 0.4% of calculated values except where otherwise noted.
b) C: Calcd, 62.07; Found, 61.46.

Biological Results and Discussion

Tables I and II show the pharmacological data obtained in the histamine release (HR) inhibition test and the rat passive cutaneous anaphylaxis (PCA) test for new compounds and established drugs (compound **1a** and disodium cromoglycate).

HR inhibitory activity was measured after a 15-min pretreatment. A few of the 4-(4-carboxybenzoyl) compounds **2m**, **2n** and **12** show some degree of inhibitory activity on histamine release. None of these compounds showed any potentiation of the release. The other compounds can be regarded as inactive, with the exception of **2j**. Compound **2j** was as potent as **2n**. Surprisingly, the 6,7-methylenedioxy derivatives had no appreciable activity (see Table II).

Anti-PCA activity was measured 3 h after *i.p.* administration. It is clear that some isoquinolones (**2b**, **2k** and **2n**) showed an inhibitory effect on the PCA reaction. Compounds **2b** and **2k** having one hydrogen on the amino group at position 2 must be converted *in vivo* to an active form, since they showed no significant effect in the *in vitro* HR inhibition test. The converse findings were observed with compounds **2j**, **2m** and **12**.

Thus, although it was possible to prepare 1(2*H*)-isoquinolone analogues without any potentiating activity on histamine release but with inhibitory activity, we were not able to prepare an analogue which showed any improvement in potency. However, it is interesting that compound **2n** showed potent activity in both tests.

Experimental

Melting points were determined on a Mettler FP1 melting point apparatus with a recorder and are uncorrected. Where indicated by the symbols for the elements, the analytical values were within ±0.4% of the theoretical values. Infrared (IR) spectra were recorded on a Hitachi 215 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained with tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL JMS-D300 spectrometer.

Analytical data and mass spectra for the products are listed in Table III.

4-Acetyl-1(2*H*)-isoquinolone (2b)——AcOH (10 ml) was added to a mixture of 4-acetylisocoumarin[6a] (0.7 g, 3.7 mmol) and $(NH_4)_2CO_3$ (2.0 g, 21 mmol), and the whole was refluxed for 3 h with stirring. The reaction solution was poured into ice water (100 ml). The precipitate was collected by filtration and recrystallized from $CHCl_3$–EtOH to afford 0.37 g (52.9%) of colorless needles.

TABLE III. Analytical Values and Mass Spectra

Compd.	Formula	Calcd			Found			MS (M^+), *m/e*
		C	H	N	C	H	N	
2a	$C_{10}H_7NO_3$	63.49	3.73	7.40	63.15	3.68	7.23	189
2b	$C_{11}H_9NO_2$	70.58	4.85	7.48	70.41	4.85	7.47	187
2c	$C_{13}H_{11}NO_4$	63.67	4.52	5.71	63.55	4.44	5.71	245
2d	$C_{12}H_{11}NO_2$	71.62	5.51	6.96	71.56	5.42	6.98	201
2e	$C_{13}H_{13}NO_2$	72.54	6.08	6.51	72.54	6.05	6.45	215
2f	$C_{15}H_{17}NO_2$	74.05	7.04	5.76	73.99	7.01	5.66	243
2g	$C_{18}H_{15}NO_2$	77.96	5.45	5.05	78.11	5.38	4.94	277
2h	$C_{17}H_{13}NO_2$	77.55	4.98	5.32	77.60	5.00	5.24	263
2i	$C_{13}H_{11}NO_2$	63.67	4.52	5.71	63.44	4.55	5.69	245
2j	$C_{16}H_{20}N_2O_2 \cdot HCl \cdot 1/2H_2O$	60.47	6.98	8.81	60.87	6.96	8.73	272
2k	$C_{17}H_{11}NO_4$[a]	69.62	3.78	4.78	69.04	3.92	5.04	283
2m	$C_{18}H_{13}NO_4$	70.35	4.26	4.56	69.98	4.22	4.54	307
2n	$C_{21}H_{19}NO_4 \cdot CH_3OH$	69.28	6.28	3.67	69.30	6.02	3.75	349
4	$C_{11}H_{11}ClO_5$	51.08	4.29	—	49.86	4.34	—	252
6	$C_{12}H_8O_5$[a]	62.07	3.47	—	61.46	3.46	—	232
7a	$C_{12}H_9NO_4$	62.34	3.92	6.06	61.99	3.94	6.03	231
7b	$C_{13}H_{11}NO_4$	63.67	4.52	5.71	63.32	4.55	5.64	245
9	$C_{11}H_7ClO_3$	59.35	3.19	—	59.31	3.16	—	222
10	$C_{17}H_{11}BrO_3$	59.50	3.23	—	59.53	3.16	—	342
11	$C_{18}H_{11}NO_3$	74.73	3.83	4.84	74.49	3.81	4.85	289
12	$C_{18}H_{12}O_5$	70.13	3.92	—	69.79	3.95	—	308
1a	$C_{17}H_{10}O_5$	69.39	3.43	—	69.10	3.31	—	294

a) Analysis value for C differed by more than ±0.4% from the calculated value.

The other compounds (**2c**—**n** and **7a**, **b**) were prepared by the reaction of **1** or **6** with primary amines according to the same procedure as described for **2b**.

4-Chloroformyl-6,7-methylenedioxyisocoumarin (**4**)——A mixture of **3** (23.5 g, 0.1 mol) and $SOCl_2$ (72 g, 0.6 mol) in toluene (50 ml) in the presence of a catalytic amount of pyridine was heated at 80—90°C for 6 h with stirring, then cooled. The resultant precipitate was collected by filtration, and recrystallized from toluene to afford 19.6 g (75.8%) of colorless needles, mp 208—209°C.

4-Acetyl-6,7-methylenedioxyisocoumarin (**6**)——A solution of diethyl malonate (2.28 g, 17 mmol), anhydrous EtOH (1.6 ml) and C_6H_5Cl (2 ml) was added dropwise to a mixture of Mg (0.43 g, 18 matm), anhydrous EtOH (0.4 ml) and CCl_4 (0.1 ml). After the initial vigorous reaction had subsided, the mixture was heated at 80°C for 1 h. A mixture of **4** (4.12 g, 16.3 mmol) and C_6H_5Cl (30 ml) was added dropwise to the reaction mixture (maintained at 27—33°C) with stirring, and the whole was then stirred for 2 h. Acidification of the mixture with 20% H_2SO_4 (10 ml) gave a precipitate, which was collected by filtration. The precipitate was refluxed in a solution of H_2SO_4 (10 ml), AcOH (20 ml) and water (20 ml) for 3 h, then the mixture was poured into ice water (300 ml). The resultant precipitate was collected by filtration, and recrystallized from $CHCl_3$ to yield 1.4 g (52.8%) of colorless needles.

4-Chloroformyl-3-methylisocoumarin (**9**)——Compound **9** was prepared from **8** by the same method as described for **4**, mp 82—83.5°C (recrystallized from toluene).

4-(4-Bromobenzoyl)-3-methylisocoumarin (**10**)——Pulverized anhydrous $AlCl_3$ (16.5 g, 124 mmol) was added to a solution of **9** (11 g, 49 mmol), C_6H_5Br (11 g, 70 mmol) and tetrachloroethane (55 ml), and the mixture was heated at 90—100°C for 3 h with stirring, then poured into ice water (300 ml). The excess C_6H_5Br and solvent were removed by steam distillation, and the residue was extracted with $CHCl_3$. The extract was concentrated under reduced pressure, and the residue was recrystallized from hexane (charcoal) to yield 8.5 g (50.3%) of colorless needles, mp 123.5—125°C.

4-(4-Cyanobenzoyl)-3-methylisocoumarin (**11**)——A mixture of **10** (6.4 g, 18.7 mmol), CuCN (2.5 g, 28.1 mmol) and DMF (60 ml) was heated at 160—165°C for 5 h with stirring, then cooled. A solution of $FeCl_3$ (34 g), conc. HCl (6 ml) and water (36 ml) was added to the reaction mixture, and the whole was stirred at 60—70°C for 20 min. The mixture was poured into water and the resultant precipitate was collected by filtration, and recrystallized from EtOH–acetone to afford 4.35 g (69.8%) of pale yellow needles, mp 190.5—191.5°C.

4-(4-Carboxybenzoyl)-3-methylisocoumarin (**12**)——A mixture of **11** (4.0 g, 13.8 mmol), AcOH (20 g), H_2SO_4 (20 g) and water (20 ml) was heated at 130—135°C for 4 h with stirring. The mixture was poured into ice water (400 ml) and the resultant precipitate was collected by filtration and dissolved in 5% $NaHCO_3$ (200 ml). Undissolved material was removed by filtration. The filtrate was acidified, and the resultant precipitate was collected by filtration, and recrystallized from EtOH to yield 1.7 g (39.9%) of yellow plates.

Biological Methods——The antiallergic properties of the compounds were estimated by means of the inhibition test of mannan-induced histamine release (HR) from rat mast cells as previously described[3] and by means of the passive cutaneous anaphylaxis (PCA) test in rats as follows. The immunogen used was obtained by mixing 2,4-dinitrophenyl (DNP) sulfonic acid (Na-salt) and keyhole limpet hemocyanin (KLH).[11] The DNP-coupled KLH contained 12 DNP groups per KLH molecule. Rat anti-DNP-KLH serum containing IgE was elicited in male Sprague-Dawley rats (180—200 g) by injecting 1 mg of the immunogen into the footpads and 2×10^{10} heat-killed *Bordetella pertussis* organisms *i.p.* on day 0. On day 5, the animals were boosted *i.m.* with 1 mg of DNP-KLH and on day 12 they were bled by cardiac puncture under light ether anesthesia. The serum was pooled and frozen until use. Male Sprague-Dawley rats (200 g) were passively sensitized with this serum by injection (*i.d.*) of 0.1 ml of a 1: 10 dilution into the shaved dorsal surface. After a 72-h latent period, the animals were challenged *i.v.* with 1.0 ml of a 0.1% solution of DNP-KLH containing 5 mg of Evans blue. Thirty minutes later, the animals were sacrificed, and the dorsal skin was removed to measure colorimetrically the amount of exudate dye.[12] Drugs were administered *i.p.* 3 h before antigen challenge.

In order to estimate the median inhibitory dose (ID_{50}), regression lines were obtained by using at least three log-spaced doses in duplicate with four rats for each dose. The ID_{50} value was obtained by the method of inverse prediction. For the evaluation of the HR inhibitory activity, the median inhibitory concentration (IC_{50}) and its 95% confidence range were calculated.

Acknowledgement We thank Ms. Iyoko Gyounouchi for the preparation of the manuscript.

References

1) M. Kimura, I. Waki and M. Kokubo, *Jpn. J. Pharmacol.*, **28**, 693 (1978).
2) K. Nitta, J. Imai, I. Yamamoto and Y. Yamamoto, *Agric. Biol. Chem.*, **27**, 817 (1963).
3) I. Waki, Y. Deguchi and M. Kimura, *Jpn. J. Pharmacol.*, **31**, 627 (1981).
4) S.I. Kanevskaya, I.N. Kovsharova and L.I. Linevik, *Stalei Obschchei Khim*, **2**, 1493 (1953); *idem*, *Chem. Abstr.*, **49**, 5478 (1955).

5) S. Nagahara, Y. Deguchi, M. Kimura and I. Waki, *Contribution from the Research Group on Atoms and Molecules*, **15**, 104 (1979).
6) *a*) M. Kimura and M. Kokubo, Japan. Kokai Tokkyo Koho, 108072 (1976) [*Chem. Abstr.*, **86**, 155512*t* (1977)]; *b*) M. Kokubo and M. Kimura, Japan. Patent 8035 (1967) [*Chem. Abstr.*, **67**, 116819*m* (1967)]; *c*) M. Kokubo and M. Kimura, Japan. Patent 8037 (1967) [*Chem. Abstr.*, **67**, 116821*f* (1967)].
7) V.H. Belgaonkar and R.N. Usgaonkar, *J. Indian Chem. Soc., Perkin Trans. 1*, **1977**, 702.
8) R.B. Tirodar and R.N. Usgaonkar, *J. Indian Chem. Soc.*, **48**, 192 (1971).
9) F. Eiden and B.S. Nagar, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.* **297**, 488 (1964).
10) H. Tomisawa and R. Fujita, *Chem. Pharm. Bull.*, **21**, 2585 (1973).
11) H.N. Eisen, S. Belman and M.E. Carsten, *J. Am. Chem. Soc.*, **75**, 4583 (1953).
12) V.L. Beach and B.G. Steinetz, *J. Pharmacol. Exp. Ther.*, **131**, 400 (1961).