Journal of Medicinal and Pharmaceutical Chemistry

VOL. 3, No. 3 (1961)

N-Substituted 8-Aminomethylchromones, a new Class of Central Nervous System Stimulants

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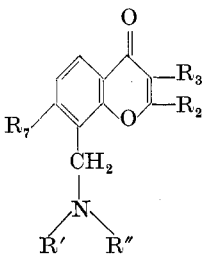
Introduction

The observation[1] that high doses of *N*-substituted 6-aminomethylchromones or flavones provoke convulsions due to excitation of the central nervous system (CNS) has led us to start a more detailed study on the importance of the aminomethyl radical combined with the chromone or flavone molecule. The chemical and pharmacological screening of various derivatives has shown that the CNS stimulant activity increases considerably when the aminomethyl radical is in the 8-position of the chromone nucleus and the compounds have a structural formula (I).

(I)



R_2 = H, CH_3, C_2H_5, or C_6H_5
R_3 = H, CH_3 or C_2H_5
R_7 = OH, OCH_3, OC_2H_5, OC_3H_7, OC_4H_9
R′,R″ = H, CH_3, C_2H_5, $(CH_2)_5$, $(CH_2)_2O(CH_2)_2$
$(CH_2)_2NCH_3(CH_2)_2$, $C_6H_5CH_2$

It has further been noted that the most active products stimulate specifically the brain stem and thus, pharmacologically, they can be classed together with pentylenetetrazol, bemegride and picrotoxin, although the new compounds do not bear a chemical likeness to these brain stem stimulants.

The chemical data and the synthesis of some of the products have been published elsewhere,[2, 3] this report being particularly concerned with the structure–activity relationship of this new class of brain stem stimulants and with their pharmacological activities.

Methods

Acute toxicity. Graduated doses of the substances under investigation were injected intraperitoneally into albino mice. The LD_{50}'s were calculated by Litchfield and Wilcoxon's method[4] from the mortality rate observed during a 24-hour period. For the majority of the compounds, the LD_{50}'s were assessed approximately on groups of 20–40 animals. Some of the substances were administered to a greater number of animals (60–100) in order to get a more accurate evaluation of the LD_{50}, and in these cases the fiducial limits are given ($P = 0 \cdot 05$).

CNS stimulant activity. All the new products, in toxic doses, cause severe clonic convulsions, sometimes followed by maximal tonic extensions, these being typical effects of brain stem stimulants such as pentylenetetrazol[5] and bemegride.[6] Indeed, the paroxysmal excitation of the CNS provoked by these substances is the principal cause of death of the intoxicated animals. For this reason, the reciprocal value of the LD_{50} of the compounds may be considered a fairly good index of the stimulant activity on the CNS. Since all the products were compared to pentylenetetrazol, their potency ratio to this brain stem stimulant regarding the CNS stimulating activity was calculated on the basis of the reciprocal values of the LD_{50}'s.

Effects upon blood pressure and on respiration. These effects were tested on rats, injecting the substances intravenously in doses equivalent to $0 \cdot 1$ of the intraperitoneal LD_{50} for mice. The arterial pressure was recorded from the incannulated carotid by a mercury manometer. A variation in pressure lasting less than 10 min after the injection and not exceeding 20 mm of Hg was defined as slight; a variation lasting less than 5 min and not exceeding 10 mm of Hg was defined as *nil.*

From the incannulated trachea, the pneumotachogram was also recorded. An increase in ventilation lasting more than 20 min was classified as $++$, an increase lasting less than 20 min was

classified as +, and when no increase in ventilation was observed the classification was 0.

These experiments were performed only with the substances which, at the dose previously indicated, could be dissolved in less than 0·5 ml of a 0·9 per cent NaCl solution and hence were easily injectable.

Inotropic effect upon the heart. This was tested on rabbit hearts isolated by the Langendorff method and perfused according to a technique previously described.[7] A positive inotropic activity was regarded as present when a substance, at a concentration of 5 mg/l., was capable of increasing the height of the cardiac contractions by more than 10 per cent in two preparations for at least 20 min.

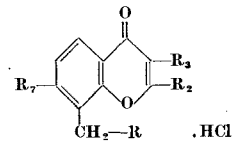
Analeptic activity. This was taken as the capacity of a product to save from death mice intoxicated by lethal doses of pentobarbital sodium. Each day of the experiment, the intraperitoneal dose of the barbiturate which killed 99 per cent of the animals between 45 and 60 min after administration (LD_{99}) was determined. This dose, usually between 130 and 140 mg/kg, was administered to groups of 20 mice; simultaneously the substance to be tested was injected by the subcutaneous route in a dose equivalent to 2 LD_{50}. Owing to the antagonism existing between pentobarbital and these CNS stimulants, doses of up to 2–6 times the LD_{50} of the latter were still well tolerated in the barbiturate intoxicated animals.

The analeptic activity of the substances under test was regarded as zero (0) if in the 24 hours following the administration of the drugs 80–100 per cent of the animals died, as + when the survival rate was 20–60 per cent, and + + when the survival rate was over 60 per cent. The analeptic activity of pentylenetetrazol, which was also tested in each experiment, was such as to save 85–95 per cent of the mice. All experiments were carried out at a constant room temperature of $24 \pm 1°$ C.

Results and Discussion

The pharmacological results reported in Table I allow the following inferences to be made regarding the structure–activity relationships of the aminomethylchromones under examination.

Table I. Structure and pharmacological activity of CNS stimulant alkylaminomethylchromones



Compd.	R_2	R_3	R_7	R	Mol. wt.	LD_{50} mg/kg	LD_{50} μmoles/kg	CNS stimulating activity (Pentylenetetrazol = 1) compared on weight basis	CNS stimulating activity (Pentylenetetrazol = 1) compared on molar basis	Effects upon arterial pressure	Repiratory stimulant action	Inotropism	Analeptic activity	Seizure type[c]
1.	CH_3	H	OH	$N(CH_3)_2$	269·72	45	167	1·6	3·08	hypertensive	++	nil	++	c.t.
2.	CH_3	H	OH	$N(C_2H_5)_2$	297·78	70	235	1·0	2·19	slightly hypertensive	+	nil	++	c.t.
3.	CH_3	H	OH		309·79	50	161	1·4	3·19	slightly hypertensive	+·+	nil	++	c.t.
4.	CH_3	H	OH		311·76	100	321	0·7	1·60	nil	0	positive	++	c.t.
5.	H	CH_3	OH	$N(CH_3)_2$	269·72	70	260	1·0	1·98	hypertensive	++	nil	++	c.t.
6.	H	CH_3	OH	$N(C_2H_5)_2$	297·78	150	504	0·5	1·02	hypertensive	++	nil	++	c.t.
7.	H	CH_3	OH		309·79	200	646	0·4	0·80	hypertensive	+	nil	0	c.t.
8.	H	CH_3	OH		311·76	150	481	0·5	1·07	slightly hypertensive	+	nil	+	c.t.
9.	CH_3	CH_3	OH	$NHCH_3$	269·50	40	148	1·8	3·47	nil	0	nil	+	c.t.
10.	CH_3	C_2H_5	OH	$NHCH_3$	283·75	100	352	0·7	1·46	hypertensive	+	nil	+	c.t.
11.	C_2H_5	CH_3	OH	$NHCN_2$	283·75	50	176	1·4	2·92	hypertensive	+	positive	++	c.t.
12.	CH_3	C_2H_5	OH	NHC_6H_5	345·82	2500	7229	0·03	0·07	[a]	[a]	positive	[b]	c.t.
13.	CH_3	C_2H_5	OH	$NHCH_2C_6H_5$	359·84	100	278	0·7	1·85	[a]	[a]	nil		c.t.
14.	C_2H_5	C_2H_5	OH	$NHCH_3$	297·78	70	235	1·0	2·19	nil	0	nil	+	c.t.
15.	C_6H_5	CH_3	OH	$NHCH_3$	331·79	50	151	1·4	3·40	nil	0	nil	+	c.t.
16.	C_6H_5	C_2H_5	OH	$NHCH_3$	345·82	180	521	0·4	0·99	[a]	[a]	nil	0	c.
17.	C_6H_5	C_2H_5	OH	NHC_2H_5	359·84	230	639	0·3	0·80	[a]	[a]	nil	[b]	c.t.

18.	CH_3	CH_3	OH	$N(CH_3)_2$	283·74	10 (11·2–8·87)	35	7·1	14·69	hypertensive	++	positive	++	c.t.
19.	CH_3	CH_3	OH	$N(C_2H_5)_2$	311·80	20	64	3·5	8·03	hypertensive	+	nil	++	c.t.
20.	CH_3	CH_3	OH		323·80	65	201	1·1	2·56	hypertensive	+	positive	++	c.t.
21.	CH_3	CH_3	OH		325·78	55	169	1·3	3·04	hypertensive	+	nil	++	c.t.
22.	CH_3	C_2H_5	OH	$N(CH_3)_2$	297·77	25·7 (26–25)	86	2·8	5·98	hypertensive	+	nil	++	c.t.
23.	CH_3	C_2H_5	OH	$N(C_2H_5)_2$	325·82	100	307	0·7	1·67	hypertensive	+	nil	++	c.
24.	CH_3	C_2H_5	OH		337·84	75	222	0·9	2·32	[a]	[a]	nil	+	c.t.
25.	CH_3	C_2H_5	OH		339·81	100	294	0·7	1·75	nil	0	nil	0	c.t.
26.	C_2H_5	H	OH	$N(CH_3)_2$	284·75	10	35	7·1	14·69	hypertensive	0	nil	+	c.t.
27.	C_2H_5	H	OH	$N(C_2H_5)_2$	311·80	60	192	1·2	2·68	hypertensive	0	nil	++	c.t.
28.	C_2H_5	H	OH		323·80	30	93	2·3	5·53	hypertensive	+	nil	++	c.t.
29.	C_2H_5	H	OH		325·78	70	215	1·0	2·39	hypertensive	++	nil	++	c.t.
30.	C_2H_5	CH_3	OH	$N(CH_3)_2$	297·72	9	30	7·9	17·13	hypertensive	+	positive	++	c.t.
31.	C_2H_5	CH_3	OH	$N(C_2H_5)_2$	325·82	40	123	1·8	4·18	hypertensive	+	nil	++	c.t.
32.	C_2H_5	CH_3	OH		337·84	55	163	1·3	3·15	hypertensive	+	positive	++	c.t.
33.	C_2H_5	CH_3	OH		339·81	50	147	1·4	3·50	hypertensive	+	nil	+	c.t.
34.	C_2H_5	C_2H_5	OH	$N(CH_3)_2$	311·80	22·7 (23·4–22·0)	73	3·1	7·04	[a]	[a]	nil	++	c.t.
35.	C_2H_5	C_2H_5	OH	$N(C_2H_5)_2$	339·88	45	132	1·6	3·89	nil	0	positive	+	c.t.
36.	C_2H_5	C_2H_5	OH		351·87	75	213	0·9	2·41	hypertensive	+	nil	0	c.t.
37.	C_2H_5	C_2H_5	OH		353·84	270	763	0·3	0·67	hypertensive	++	nil	+	c.t.
38.	C_6H_5	H	OH	$N(CH_3)_2$	331·79	13·1 (14·2–11·9)	40	5·4	12·85	hypertensive	+	positive	++	c.t.
39.	C_6H_5	H	OH	$N(C_2H_5)_2$	359·84	10	28	7·1	18·36	hypertensive	+	positive	+	c.t.
40.	C_6H_5	H	OH		371·85	45	121	1·6	4·25	hypertensive	++	positive	++	c.

Table I—*continued*

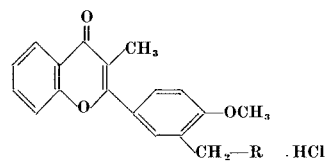
Compd.	R_2	R_3	R_7	R	Mol. wt.	LD_{50} mg/kg	LD_{50} μmoles/kg	CNS stimulating activity (Pentylenetetrazol = 1) compared on weight basis	CNS stimulating activity (Pentylenetetrazol = 1) compared on molar basis	Effects upon arterial pressure	Respiratory stimulant action	Inotropism	Analeptic activity	Seizure type[c]
41.	C_6H_5	H	OH		373·83	50	134	1·4	3·84	hypertensive	++	nil	+·+	c.
42.	C_6H_5	CH_3	OH	$N(CH_3)_2$	345·80	8·0 (8·7–7·4)	23	8·9	22·35	hypertensive	+	positive	++·	c.t.
43.	C_6H_5	CH_3	OH	$N(C_2H_5)_2$	373·85	35	94	2·0	5·47	hypertensive	++	positive	+·+·	c.
44.	C_6H_5	CH_3	OH		385·88	120	311	0·6	1·65	nil	0	nil	0	c.
45.	C_6H_5	CH_3	OH		387·84	Non-stable in solution								
46.	C_6H_5	C_2H_5	OH	$N(CH_3)_2$	359·84	34·8 (38–32)	97	2·0	5·30	nil	0	positive	+	c.
47.	C_6H_5	C_2H_5	OH	$N(C_2H_5)_2$	387·90	120	309	0·6	1·66	[a]	[a]	nil	[b]	c.
48.	C_6H_5	C_2H_5	OH		399·81	250	625	0·3	0·82	[a]	[a]	nil	[b]	c.t.
49.	C_6H_5	C_2H_5	OH		401·88	2500	6221	0·03	0·08	[a]	[a]	nil	[b]	no convulsions
50.	H	C_6H_5	OH	$N(CH_3)_2$	331·79	50	151	1·4	3·40	slightly hypertensive	++	nil	+	c.t.
51.	H	C_6H_5	OH	$N(C_2H_5)_2$	359·84	100	278	0·7	1·85	hypertensive	0	nil	+	c.t.
52.	H	C_6H_5	OH		371·85	60	161	1·2	3·19	hypertensive	+·+	nil	+	c.t.
53.	H	C_6H_5	OH		373·83	2300	6153	0·03	0·08		[a]	nil	[b]	
54.	CH_3	C_6H_5	OH	$N(CH_3)_2$	345·80	20	58	3·5	8·86	slightly hypertensive	++	nil	+·+	c.t.
55.	CH_3	C_6H_5	OH	$N(C_2H_5)_2$	373·85	50	134	1·4	3·84	hypertensive	+	nil	+·+	c.t.

56.	CH_3	C_6H_5	OH		385·88	20	52	3·5	9·88	slightly hypertensive	+	nil	+ +	c.t.
57.	CH_3	C_6H_5	OH		387·84	80	206	0·9	2·50	slightly hypertensive	+ +	nil	+	c.t.
58.	CH_3	CH_3	OCH_3	NH_2	269·72	80	297	0·9	1·73	*a*	*a*	nil	*b*	c.t.
59.	C_6H_5	CH_3	OCH_3	NH_2	331·79	50	151	1·4	3·40	nil	0	nil	0	c.
60.	CH_3	CH_3	OCH_3	$NHCH_3$	283·75	20	71	3·5	7·24	nil	+	nil	+	c.t.
61.	C_6H_5	CH_3	OCH_3	$NHCH_3$	345·82	50	145	1·4	3·54	nil	0	nil	0	c.
62.	C_6H_5	C_2H_5	OCH_3	NHC_2H_5	337·84	120	355	0·6	1·45	nil	0	nil	0	c.
63.	C_6H_5	C_2H_5	OCH_3	NHC_6H_5	421·91	>2500	—	<0·03		*a*		positive	*b*	no convulsions
64.	C_6H_5	C_2H_5	OCH_3	$NHCH_2C_6H_5$	435·83	250	574	0·3	0·90	*a*	*a*	nil	*b*	c.t.
65.	CH_3	CH_3	OCH_3	$N(CH_3)_2$	297·78	3·3 (3·6–2·0)	11	21·5	46·73	slightly hypertensive	+ +	positive	+ +	c.t.
66.	CH_3	CH_3	OCH_3	$N(C_2H_5)_2$	325·83	13 (14–12)	40	5·5	12·85	slightly hypertensive	+ +	nil	+ +	c.t.
67.	CH_3	CH_3	OCH_3		337·84	17 (18–15)	50	4·2	10·28	slightly hypertensive	+ +	nil	+ +	c.t.
68.	CH_3	CH_3	OCH_3		339·81	17·4 (18–16)	50	4·1	10·08	hypertensive	+ +	positive	+ +	c.t.
69.	CH_3	CH_3	OCH_3		389·32	150	385	0·5	1·34	slightly hypertensive	0	nil	0	c.t.
70.	CH_3	C_2H_5	OCH_3		351·87	50	142	1·4	3·62	slightly hypertensive	+	nil	+	c.
71.	CH_3	C_2H_5	OCH_3		353·84	65	184	1·3	2·79	nil	+	nil	+ +	c.
72.	C_2H_5	CH_3	OCH_3	$N(C_2H_5)_2$	339·86	20	59	3·5	8·71	hypertensive	+	nil	+ +	c.t.
73.	C_2H_5	CH_3	OCH_3		353·84	17	48	5·9	10·71	nil	+	nil	+ +	c.t.
74.	C_2H_5	C_2H_5	OCH_3		365·89	40	109	1·8	4·72	nil	+ +	nil	+	c.t.
75.	C_2H_5	C_2H_5	OCH_3		367·87	65	177	1·3	2·90	slightly hypertensive	+	positive	+ +	c.
76.	C_6H_5	CH_3	OCH_3	$N(CH_3)_2$	359·24	4·8 (5·1–4·6)	13	14·8	39·54	nil	+ +	nil	+ +	c.t.

Table I—*continued*

Compd.	R_2	R_3	R_7	R	Mol. wt.	LD_{50} mg/kg	LD_{50} μmoles/kg	CNS stimulating activity (Pentylenetetrazol = 1) compared on weight basis	CNS stimulating activity (Pentylenetetrazol = 1) compared on molar basis	Effects upon arterial pressure	Respiratory stimulant action	Inotropism	Analeptic activity	Seizure type[c]
77.	C_6H_5	CH_3	OCH_3	$N(C_2H_5)_2$	387·90	28 (29–26)	72	2·5	7·14	nil	+	nil	+	c.
78.	C_6H_5	CH_3	OCH_3		399·90	55 (58–51)	138	1·3	3·73	nil	+	nil	0	c.
79.	C_6H_5	CH_3	OCH_3		401·87	85	212	0·8	2·42	nil	+	positive	+	c.t.
80.	C_6H_5	CH_3	OCH_3		451·39	70	155	1·0	3·32	nil	0	nil	0	c.
81.	C_6H_5	C_2H_5	OCH_3	$N(CH_3)_2$	373·87	20	54	3·5	9·52	nil	0	nil	0	c.
82.	C_6H_5	C_2H_5	OCH_3	$N(C_2H_5)_2$	401·92	60	149	1·2	3·45			nil	[b]	c.
83.	C_6H_5	C_2H_5	OCH_3		413·93	550	1329	0·1	0·39	nil	0	nil	0	c.t.
84.	C_6H_5	C_2H_5	OCH_3		415·91	250	601	0·3	0·86		[a]	nil		c.t.
85.	CH_3	CH_3	OC_2H_5	$N(CH_3)_2$	311·82	5	16	14	32·13	hypertensive	+ +	nil	+ +	c.t.
86.	C_6H_5	CH_3	OC_2H_5	$N(CH_3)_2$	373·87	18	48	3·9	10·71	nil	+	nil	+	c.t.
87.	C_6H_5	CH_3	O-i-C_3H_7	$N(CH_3)_2$	387·89	30	77	2·4	6·68	slightly hypotensive	+	nil		c.
88.	C_6H_5	CH_3	OC_4H_9	$N(CH_3)_2$	401·92	40	100	1·8	5·14	hypotensive	0	nil	0	c.
		Pentylentetrazol			138·18	71 (80–63)	514	1	1	slightly hypertensive	+ +	nil	+ +	c.t.

Table I—*continued*



Compound	R	Mol. wt.	LD_{50}		CNS stimulating activity (Pentylenetetrazol = 1) compared on		Effects upon arterial pressure	Respiratory stimulant action	Inotropism	Analeptic activity	Seizure type[c]
			mg/kg	μmoles/kg	weight basis	molar basis					
89.	$N(C_2H_5)_2$	387·89	170	438	0·4	1·17	nil	0	nil	0	c.
90.		399·91	150	375	0·5	1·37	hypotensive	0	nil	0	c.
91.		401·38	332	827	0·2	0·62	nil	+	nil	0	c.t.

[a] The water-solubility was too low for intravenous administration.
[b] The water-solubility was lower than 1‰ and therefore no proof of analeptic activity was performed.
[c] c. = clonic convulsions; c.t. = clonic convulsions followed by maximal tonic extension.

CNS Stimulant Activity

(*a*) *Radicals in the 2-position of the chromone nucleus.* The products tested had either a hydrogen, a methyl, an ethyl or a phenyl radical in the 2-position. Taking the average of the activities of the substances which differed only as regards the substituent in the 2-position, it was found that the ethyl radical conferred the greatest CNS stimulating potency. The activity of the 2-methylchromone homologues was on the average only slightly less (1·06 times), while the 2-phenylchromone derivatives gave an activity which was 1·6 times less than that of the analogous 2-ethylchromones.

(*b*) *Radicals in the 3-position.* Without exception, the products with a methyl radical in the 3-position were more potent than those with an ethyl radical in the same position. On the average, the activity ratio of the two groups of substances was as 2:1. The activity of those substances which had no substituent in the 3-position was on the average 1·3 times greater than that of the homologues in which the 3-position was occupied by a methyl, but this increase in activity due to the lack of a methyl radical in the 3-position seemed not to be constant.

(*c*) *Radicals in the 7-position.* The methoxy radical confers on the derivatives an activity which is on the average 2 times higher than that of the corresponding 7-ethoxychromones or the 7-hydroxychromones. The only exception to this rule was observed with the 7-hydroxy- and 7-methoxy-2-phenyl-3-ethyl-8-piperidinomethylchromones (Nos. 48 and 83 of Table I). The homologues with a 7-isopropyloxy or butoxy chain seem to be less active.

(*d*) *Radicals in the 8-position.* It was possible to compare the products differing in the following radicals: 8-aminomethyl-, 8-methylaminomethyl-, 8-dimethylaminomethyl-, 8-ethylaminomethyl-, 8-diethylaminomethyl-, 8-piperidinomethyl-, 8-morpholinomethyl-, 8-*N*-methylpiperazinomethyl-, and 8-benzylaminomethyl. Without exception, the most active of all proved to be the 8-dimethylaminomethylchromones, which were, on the average, twice as active as the 8-diethylaminomethyl homologues, *ca.* 3 times more active than the 8-methylaminomethyl homologues and the 8-piperidinomethylchromones, and 4–5 times more

active than the 8-morpholinomethyl-, the 8-benzylaminomethyl- and the 8-ethylaminomethylchromones. Still less active were the 8-aminomethylchromones and the 8-*N*-methylpiperazinomethylchromones. These relationships between the nature of the various substituents and the CNS stimulant activity of the compounds are summarized in Table II.

The most active product of the series was No. 65, i.e. 2,3-dimethyl-7-methoxy-8-dimethylaminomethylchromone (Rec 7–0268), which showed about the same CNS stimulating activity as picrotoxin (Table III), up to now considered as the most potent brain stem stimulant. Evidently the new series of *N*-substituted 8-aminomethylchromones includes substances of enormous CNS stimulant activity; indeed some compounds such as, for example, Rec 7–0268 and No. 76 (3-methyl-7-methoxy-8-dimethylaminomethylflavone) (Rec 7–0267), as well as 5,7-diphenyl-1,3-diazadamantan-6-ol, a spinal cord stimulant described by Longo *et al.*,[8] probably represent the most potent synthetic CNS excitants so far described.

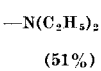
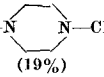
To obtain this extraordinary activity, the aminomethyl chain must be located in the 8-position. In fact, investigators who have described chromones with this chain in the 2-position[9,10] or in the 3-position[11] have found products with antispasmodic, coronary vasodilator and analgesic properties but no CNS stimulant activity. If this aminomethyl chain is in the 6-position, one can obtain chromones which, at high doses, are already convulsant.[1] In fact, it was this observation that provided the starting point for the systematic research here described.

The methoxyl or hydroxyl group in the 7-position seems to be less important, for several authors, including Wiley[11] and Jongebreur,[9] have described 7-hydroxy- or -methoxychromones without ever noting any CNS stimulant activity.

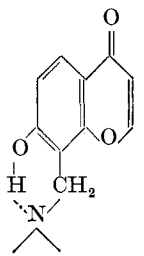
Effect upon Blood Pressure

Changes in blood pressure are less easily translated into quantitative terms than the CNS stimulating activity and therefore the discussion of the relationships between the structure of the substances under study and their effect upon the arterial pressure becomes more difficult. However, it can be noted that substances with a hydroxyl group in the 7-position are almost always

Table II. Relation between the CNS stimulant activity and the type of substituent in the chromone molecule

	Activity sequence (average activity, expressed as a percentage, is in brackets)
R_2	$-C_2H_5$ (100%) > $-CH_3$ (99%) > C_6H_5 (62%)
R_3	$-H$ (100%) > $-CH_3$ (75%) > $-C_2H_5$ (32%)
R_7	$-OCH_3$ (100%) > $-OC_2H_5$ (52%) > $-OH$ (43%) > $-O\text{-}i\text{-}C_3H_7$ (17%) > $-OC_4H_9$ (12%)
R	$-N(CH_3)_2$ (100%) > $-N(C_2H_5)_2$ (51%) >  (32%) > $-NHCH_3$ (29%) > $-NHCH_2C_6H_5$ (23%) >  (23%) > $-NHC_2H_5$ (20%) > $-NH_2$ (14%) >  (19%)

hypertensive (38 out of the 46 substances examined from this point of view) whereas this action is less frequently found among the 7-methoxychromones (only 3 hypertensive and 6 slightly hypertensive out of the 26 substances examined). It is not unlikely that the substantially different pharmacological behaviour of chemically very similar substances is due to the fact that only the 7-hydroxychromones can form another ring through a hydrogen bridge and therefore present the structure (II). This con-



(II)

figuration resembles the one found in other classes of CNS stimulants, including some markedly hypertensive sympathomimetic amines described by several authors,[12–15] in which this cyclic structure *via* a hydrogen bridge can be postulated.[16] However, it must be mentioned that in these substances a phenylisopropylamine fragment can always be identified, which is not present in the 7-hydroxy-8-*N*-substituted-aminomethylchromones.

Effects on Respiration

The pneumotachographic recording of respiration yields only qualitative data. A discussion of the quantitative respiratory effects is therefore almost impossible. However, it may be noted that for the substances with a hydroxyl group in the 7-position there seems to be a fairly good correlation between the hypertensive and the respiratory stimulant activity. This correlation is not so good for the 7-methoxychromones. For these substances, a better correlation seems to exist between the respiratory stimulant and the analeptic activity.

Inotropic Action

Beside hypertensive activity, the 7-hydroxychromones tend to show also a positive inotropic action (14 out of the 56 substances

investigated), while the 7-methoxychromone derivatives show this property less frequently (5 out of 31 substances). Here again it is not possible to establish a more precise relationship between the chemical structure and the pharmacological activity.

Analeptic Activity

From a practical, i.e. therapeutic, point of view, the analeptic activity is perhaps the most interesting among the pharmacological properties examined, because it represents an index of the capacity for saving animals from death due to profound depression of the medullary centres. As has been shown elsewhere,[17] this analeptic activity is a specific characteristic of the most active brain stem stimulants (e.g. picrotoxin, pentylenetetrazol, bemegride) and is not found in other CNS stimulants such as strychnine, *d*-amphetamine and nikethamide. The presence of an analeptic action gives therefore, *inter alia*, also an indication as to the mode of action of the drugs.

Most of the investigated *N*-substituted 7-hydroxy- or 7-methoxy-8-aminomethylchromones showed pronounced analeptic activity (only 14 out of the 74 substances tested failed to exhibit this property to an appreciable extent), but we were not able to establish a relationship between the chemical structure and the absence or the presence of analeptic activity. The products lose their analeptic activity if the methoxy and the *N*-substituted aminomethyl groups are located in the 4-position and the 3-position, respectively, of the phenyl in 3-methylflavone.

Types of Convulsions

As shown in Table I, the majority of the substances caused an intoxication picture characterized by clonic convulsions followed by maximal tonic extension. According to Penfield and Erickson,[18] Cheymol,[5] Goodman *et al.*,[19] and Kreindler *et al.*,[20] this symptomatology is the expression of a paroxysmal excitation of both the cortex and the subcortex. Only a limited number of the described substances gave rise solely or mainly to clonic convulsions, which, in the opinion of the quoted authors, are an expression mainly of cortical stimulation.

Incidentally, it may be noted that generally the substances

which give rise to clonic convulsions without tonic extension exert little or no analeptic activity.

In Table III, a comparison of the CNS stimulating activity of two of the most powerful chromone derivatives (No. 65 and 76) with the activity of well-known brain stem stimulants (pentylenetetrazol, bemegride and picrotoxin) is reported. Furthermore, in Table III there are also other substances with different mechanisms of action, like strychnine (mostly a spinal cord stimulant), nikethamide, prethcamide and *N*,*N*-diethyl-3-ethoxy-4-hydroxybenzamide whose mechanism of action is not yet well defined.[17] Owing to the different mechanisms of action of all of these substances, the comparison was not made on the basis of the LD_{50} as in Table I, but on the basis of the convulsant dose in 50 per cent of the animals (CD_{50}), which is more strictly related to the CNS stimulating activity.

Table III shows that the chromone derivatives are more powerful than all the other synthetic compounds. Indeed their CNS stimulating activity is of the same order of magnitude as that of picrotoxin, the most active brain stem stimulant known. It is not proper, however, to compare the chromone derivatives with picrotoxin since the mechanism of action does not seem to be the same. In fact, as shown in the last column of Table III, the duration of the picrotoxin activity is much longer than that of all the other compounds tested, this being one of the reasons why picrotoxin therapy may sometimes be dangerous as it may cause convulsions after a relatively long time following administration.

As stated earlier, analeptic activity is one of the most interesting properties for possible therapeutic use and hence researches were undertaken with the object of selecting the most promising products from this point of view. Bearing in mind certain medical criteria, i.e. that a good analeptic must take effect in small doses, must have a wide margin of safety, must exert an intense and constant stimulant action upon the respiratory centre and, finally, must not have too marked a hypertensive action in order not to be contra-indicated in hypertensive subjects, in persons with vascular rigidity or in anaesthesia during surgical operations, it was found that the substance which combined all these properties

Table III. Convulsant activity of two chromone derivatives in comparison with that of other CNS stimulants

Compound	CD_{50} i.p.,[a] mg/kg	CNS stimulating activity (Pentylenetetrazol = 1)		Time interval after administration of the drugs during which pharmacological effects (hyperexcitability, convulsions and death) were observed, min
		compared on mg/kg activity basis	compared on molar basis	
Pentylenetetrazol	40·50 (43·2–38·0)	1	1	1–10
Bemegride	14·10 (17·6–11·2)	2·87	3·22	1–8
Picrotoxin	4·80 (6·21–3·69)	8·44	36·63	6–1400
No. 76 (Rec 7–0267)	4·03 (4·39–3·70)	10·05	26·64	2–10
No. 65 (Rec 7–0268)	2·98 (3·34–2·66)	13·59	29·30	2–6
Strychnine	0·55 (0·71–0·42)	73·64	183·13	2–20
Nikethamide	145 (160–131)	0·28	0·36	4–90
Prethcamide[b]	142 (171–118)	0·29	0·48	1–90
DEHB[c]	25·50 (27·9–23·3)	1·59	2·74	1–20

[a] Convulsant dose in 50% of the mice with $P = 0·05$ fiducial limits.
[b] Prethcamide = mixture of equal parts of the dimethylamides of *N*-crotonyl-α-ethylaminobutyric acid and *N*-crotonyl-α-propylaminobutyric acid.
[c] DEHB = *N*,*N*-diethyl-3-ethoxy-4-hydroxybenzamide.

in the highest degree is No. 76 of Table I, i.e. 3-methyl-7-methoxy-8-dimethylaminomethylflavone hydrochloride (Rec 7-0267; proposed non-proprietary name: dimefline). As will be shown in a further report concerning researches conducted on rabbits, cats and dogs, this compound has a respiratory stimulant effect about 250 times greater than that of pentylenetetrazol in normal animals (rabbits) and 187 times greater in morphine-depressed animals. When mice are injected with an LD_{95} of pentobarbital (*ca.* 120 mg/kg) only 3 per cent of the animals die when treated also with 2 mg/kg of Rec 7–0267. The therapeutic index of this drug (ratio between the minimal toxic dose and the minimal active dose) is 1·5 times more favourable than that of pentylenetetrazol (17). The effects of Rec 7–0267 upon the cerebral cortex, heart and blood pressure seem negligible. Thus Rec 7–0267 (dimefline) appears as a very interesting drug for symptomatic therapy and the prevention of depressions of the brain stem and, especially, of the resulting impairment of the respiratory function.

Summary. Some of the pharmacological properties of the *N*-substituted 7-hydroxy- and 7-methoxy-8-aminomethylchromones, which represent a new class of potent brain stem stimulants, are described. The relationship between the structure and activity of these substances is considered. The most active product in the series is 2,3-dimethyl-7-methoxy-8-dimethylaminomethylchromone (Rec 7–0268), which is about as active as picrotoxin and 29 times more active than pentylenetetrazol. The most interesting substance for the purpose of therapeutic application seems to be the 3-methyl-7-methoxy-8-dimethylaminomethyl flavone (Rec 7–0267; dimefline), which has a very potent antidotal action in barbiturate poisoning, an intense respiratory analeptic action and affords a more favourable therapeutic index than any of the other brain stem stimulants which have come into medical use.

(Revised manuscript received 22 October, 1960)

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