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Central Depressant Effects of Maltol Analogs in Mice[1]

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The central actions of maltol(2-methylpyromeconic acid, II), previously isolated from the extract of *Passiflora incarnata*, and its analogs were studied in mice. 2-Butyl- and 2-isobutylpyromeconic acid (IVd) were the most potent depressants of pentetrazole-induced convulsion among the 2-alkylpyromeconic acids tested. The effect of 2-(1-hydroxy-isobutyl)pyromeconic acid (Vd) was more potent than that of IVd. Methylation of the 3-hydroxyl group of II or replacement of the O atom of the 4-pyrone ring with =NH did not alter the effect on pentetrazole-induced convulsion. Both IVd and Vd also showed depressing effects on the convulsion induced by strychnine or picrotoxin but did not affect the convulsion induced by caffeine. The anticonvulsive effects of IVd and Vd on the maximal electroshock seizure were less potent than that of 2-ethylpyromeconic acid (III). II, III, IVd and Vd depressed spontaneous motor activity, and III and Vd prolonged the hexobarbital-induced sleeping time. The brain levels of these four compounds after subcutaneous injection in mice were not related to their lipid solubilities.

Keywords——maltol analogs; anticonvulsant effect; maximal electroshock seizure; spontaneous motor activity; hexobarbital sleeping time; brain level

In the previous report[1] from our laboratory, maltol(2-methylpyromeconic acid, II) isolated from *Passiflora incarnata* dry extract was shown to have a central depressing activity in mice, and ethyl maltol(2-ethylpyromeconic acid, III) was found to be a potent anticonvulsant than II against the convulsions induced by pentetrazole and strychnine.

This paper describes studies on the relationship between the central actions of some analogs of II and their chemical structures.

Experimental

Materials——The compounds tested pharmacologically are listed in Table I.

Pyromeconic acid (I) was synthesized from meconic acid by the method of Kukolja and Hahn.[3] 2-(1-Hydroxyalkyl)pyromeconic acid (V) and 2-alkylpyromeconic acid (IV) were prepared from I by the method of Stephen *et al.*[4] The 3-methoxy compounds (VI and VII) were synthesized from I and II according to the method of Dashunin and Tovbina.[5] The preparations of 3-methoxy-6-hydroxymethyl-4-pyrone (IX) and allomaltol (X) were carried out from kojic acid (VIII) by the methods of Campbell *et al.*[6] and Yabuta,[7] respectively. Ammonolysis of VII by the method of Fisher and Hodge[8] yielded 2-methyl-3-methoxy-4-pyridone (XI), and 2-methyl-3-hydroxy-4-pyridone (XII) was obtained by hydrolysis of XI.

Pharmacological Experiments

Animal——Male ddY mice, weighing 18—22 g, were used.

Drugs——Pentetrazole (Cardiazol, Sankyo Co.), strychnine (Stchinin, Fuso Pharm. Ind.), bemegride

1) This report constitutes Part II of the series entitled "Studies on *Passiflora incarnata* Dry Extract" Part I: N. Aoyagi, R. Kimura, and T. Murata, *Chem. Pharm. Bull.*, **22**, 1008 (1974).
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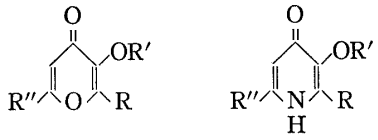
TABLE I. List of Compounds subjected to Pharmacological Tests



I—X XI, XII

No.	R	R′	R″	mp (°C) Lit.	mp (°C) Obs.[a]	Formula	Analysis (%) Calcd (Found) C	H	N
I	H	H	H	117—118	119	$C_5H_4O_3$	53.58 (53.61	3.60 3.64)	
II	CH_3	H	H	161—162	162—162.5	$C_6H_6O_3$	57.14 (—	4.80 —)	
III	C_2H_5	H	H	90— 91	91	$C_7H_8O_3$	59.99 (—	5.75 —)	
IVa	C_3H_7	H	H	84— 85	83	$C_8H_{10}O_3$	62.32 (62.30	6.54 6.60)	
IVb	C_4H_9	H	H	55— 56	53— 54	$C_9H_{12}O_3$	64.27 (64.38	7.19 7.18)	
IVc	C_6H_{13}	H	H	71— 73	74— 75	$C_{11}H_{16}O_3$	67.32 (67.38	8.22 8.20)	
IVd	$(CH_3)_2CH-CH_2$	H	H	89— 95	88— 89	$C_9H_{12}O_3$	64.27 (64.19	7.19 7.19)	
Va	$C_2H_5-CH(OH)$	H	H	140—141	139—140	$C_8H_{10}O_4$	56.46 (56.79	5.92 5.81)	
Vb	$C_3H_7-CH(OH)$	H	H	140—141	137	$C_9H_{12}O_4$	58.69 (59.10	6.57 6.45)	
Vc	$C_5H_{11}-CH(OH)$	H	H	134—135	134	$C_{11}H_{16}O_4$	62.25 (62.29	7.60 7.43)	
Vd	$(CH_3)_2CH-CH(OH)$	H	H	137—138	136—137.5	$C_9H_{12}O_4$	58.69 (58.99	6.57 6.59)	
VI	H	CH_3	H	94	93— 94	$C_6H_6O_3$	57.14 (56.93	4.80 4.92)	
VII	CH_3	CH_3	H	78— 79 /4 mmHg[b]	75— 78 /3 mmHg[b]	$C_7H_8O_3$	59.99 (59.21	5.75 5.76)	
VIII	H	H	$HOCH_2$	153—154	154.5	$C_6H_6O_4$	50.71 (—	4.26 —)	
IX	H	CH_3	$HOCH_2$	165	164—164.5	$C_7H_8O_4$	53.84 (53.91	5.16 5.04)	
X	H	H	CH_3	153	151—152	$C_6H_6O_3$	57.14 (56.95	4.80 4.86)	
XI	CH_3	CH_3	H	155—156.5	154—156	$C_7H_9O_2N$	60.42 (59.92	6.52 6.77	10.07 10.17)
XII	CH_3	H	H	>250 Decomp.	275 Decomp.	$C_6H_7O_2N$	57.59 (57.46	5.64 5.57	11.20 11.34)

a) All melting points are uncorrected.
b) bp (uncorrected).

(Antibarbi, Tanabe and Co.), picrotoxin (Wako Pure Chem. Ind.), pipradrol (Meratran, Shionogi and Co.), VIII (Wako Pure Chem. Ind.), phenobarbital (Phenobal, Fujinaga Seiyaku Co.), meprobamate (Atraxin, Daiichi Seiyaku Co.), phenytoin sodium (Aleviatin Sodium, Dainippon Pharm. Co.), and hexobarbital sodium (Ouropan Sodium, Shionogi and Co.) were used. Compounds II and III were kindly provided by Takasago Perfumery Co. and were used after recrystallization from water. All the compounds tested pharmacologically were suspended in 0.5% tragacanth solution and were administered subcutaneously to mice. The injected volume of the suspension was 0.1 ml (0.3 ml or less for the estimation of LD_{50} or ED_{50})/10 g body weight. The control group was administered an equivalent dose of 0.5% tragacanth solution.

Pentetrazole Convulsion——Thirty minutes after the injection of the test compounds, 130 mg/10 ml/kg of pentetrazole was administered intraperitoneally to mice. The times to onset of convulsion and to death

after administration of the drug were estimated. The number of animals which survived over 48 hr after the start of the experiment was counted. ED_{50} was calculated by the up and down method.[9]

Central Stimulant-induced Convulsion——Thirty minutes after the injection of the compounds, central stimulants were administered to mice by the routes and at the doses indicated in Table III. The injected volumes of the stimulant solutions were 0.1 ml/10 g body weight. After administration of the stimulant the numbers of animals which showed convulsion within 2 hr and which died within 48 hr were counted.

Electroshock Seizure——The electroshock apparatus (Kyoto Keisokuki Kogyo) designed by Woodbury and Davenport[10] was used. Thirty or sixty minutes after the administration of the compounds, the mice were shocked through corneal electrodes with a current of 25mA for 0.2sec at 60 Hz, and the number of animals which showed tonic extension was recorded. ED_{50} was calculated by the up and down method.[9]

Acute Toxicity——The compounds were administered subcutaneously to groups of 8 mice and the acute toxicity was determined with a 48 hr observation interval by the method of Litchfield and Wilcoxon.[11]

Spontaneous Motor Activity——Spontaneous motor activity in mice was determined by using an activity meter (Natsume Seisakusho TN type). Thirty minutes after the subcutaneous administration of 0.5% tragacanth solution as a control, mice were placed singly in photocell cages. The number of interruptions of the light beams caused by movement of the animals was recorded for 60 min. Twenty-four hours later, the test compounds were injected subcutaneously into the same animals and the motility was estimated again under the same conditions. All the experiments were carried out at 20—25°.

Hexobarbital-induced Sleeping Time——Thirty minutes after the administration of the test compounds, a dose of 100 mg/kg of hexobarbital sodium was injected intraperitoneally into the mice. Sleeping time was considered to be the interval between loss and recovery of an effective righting reflex. An effective righting reflex was considered to be recovery from a side position within 1 min. Control groups received the hexobarbital after injection of the vehicle alone. All the experiments were carried out at 20—25°.

Muscle Relaxation——Muscle relaxation was determined in mice by the "Rotarod" method of Dunham and Miya.[12] Thirty minutes after administering the compounds to a group of 6 mice, the animals were placed on a wooden rod, 3 cm in diameter, which was rotated at 5 rpm. The number of animals which slipped from the rod within 5 min was recorded. ED_{50} was calculated by the method of Litchfield and Wilcoxon.[11]

Solubility and Partition Coeficient——The minimum volume of the solvent required to dissolve 1 g of the compound was estimated. Organic solvent/water distribution ratios for the compounds were determined according to the method of Mayer *et al.*[13] The initial concentration of the compound in 0.1 M phosphate buffer, pH 7.4, was 100 μg/ml. The amount of the compound remaining in the aqueous phase was assayed as follows; 0.5 ml of 1% ferric ammonium sulfate in 2 N sulfuric acid was added to 5 ml of the sample solution, and the absorbancy of the ferric complex formed was determined at 520 nm (500 nm for Vd) on a Hitachi 181 spectrophotometer.

Tissue Level——Thirty or sixty minutes after the subcutaneous administration of the compounds, the plasma, liver and brain were isolated and homogenized with 5 ml of 80% ethanol. Each homogenate thus obtained was centrifuged and the supernatant was washed with 0.5 ml of heptane. A half ml of 10% ferric ammonium sulfate in 2 N sulfuric acid was added to 4 ml of the supernatant and the mixture was filtered. Colorimetric analysis of the filtrate was carried out as described above. Recoveries of the compounds from the tissues were 94—118%.

Results

1. Effect on Pentetrazole-Induced Convulsion

The effects of analogs of II on the convulsion induced by 130 mg/kg of pentetrazole in mice are shown in Fig. 1. Doses of the analog compounds were 3.96 mmol/kg, equivalent to 500 mg/kg of II. The delaying effects on the onset time of the convulsion and on the time of death increased with lengthening of the chain of the 2-alkyl substituent of I, and the 2-butyl compound (IVb) was the most potent (the mice administered IVb did not show tonic extension, but all of them died of convulsion within 12 hr). However, further lengthening of the chain of the substituent decreased the potency. The isobutyl compound (IVd) was less effective than the *n*-butyl compound (IVb). The 2-(1-hydroxy)alkyl compound (V) were more potent than the corresponding 2-alkyl compounds. Thus, compound Vd showed the

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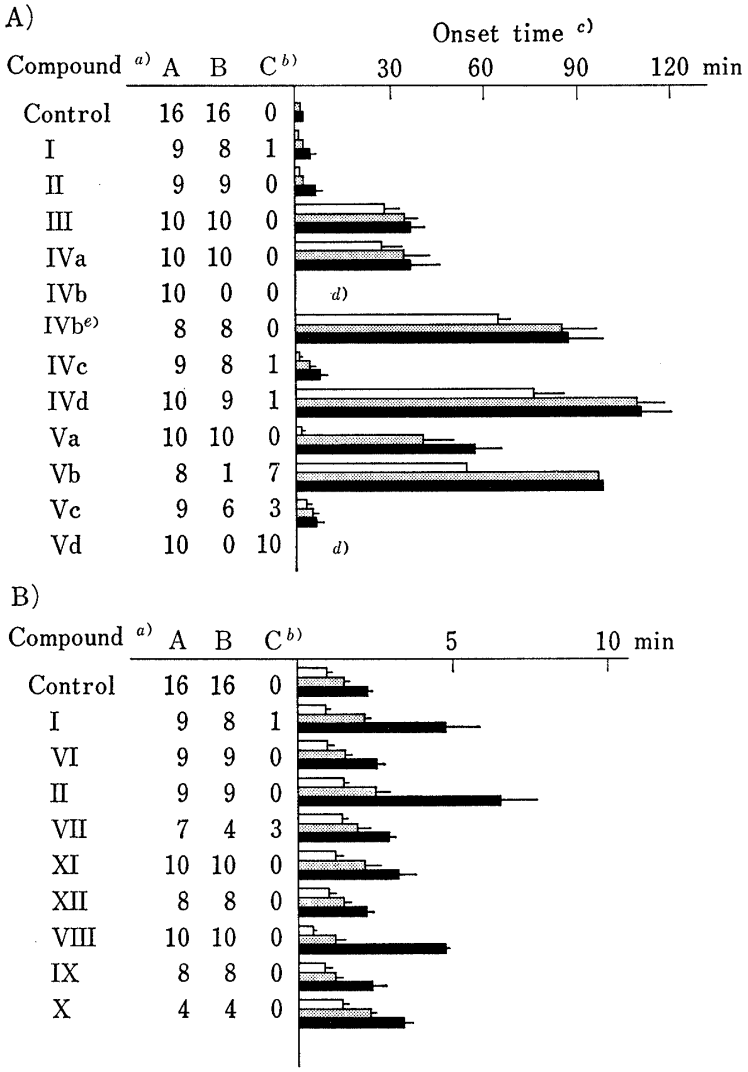

Fig. 1. Effects of Maltol Analogs on Pentetrazole (130 mg/kg *i.p.*)–Induced Convulsion in Mice

a) Dose of compounds: 3.96 mmol/kg (*s.c.*)
b) A: The number of mice used
B: The number of mice which showed convulsion and died within 3 hr
C: The number of survivals over 48 hr
c) Mean+s.e. for the mice which showed convulsion and died within 3 hr
d) No convulsion within 3 hr
e) 1.98 mmol/kg *s.c.*
□— Clonic convulsion, ▨— Tonic extension, ■— Death

TABLE II. Effects of Maltol Analogs and Anticonvulsant Agents on Tonic Extension induced by Pentetrazole (130 mg/kg *i.p.*) in Mice

Compound	Route	No.	ED_{50} (mg/kg)	ED_{50} (mmol/kg)
II	*s.c.*	9	>2000	>15.9
III	*s.c.*	9	655	4.67
IVd	*s.c.*	9	612	3.64
Vd	*s.c.*	9	515	2.80
Meprobamate	*s.c.*	9	51.7	0.237
Phenobarbital	*i.p.*	9	13.0	0.056

most potent anticonvulsant effect among the compounds tested; all the mice administered Vd survived over 48 hr without showing any convulsion. Although the number of deaths within 3 hr decreased on treatment with VII, the substitution of a methoxyl for the 3-hydroxyl group of I, II, VIII or XII, or of an =NH group for the oxygen atom of the 4-pyrone ring in II or VII was not effective in delaying convulsion. Compounds VIII and X showed very weak anticonvulsant effect, like II.

The ED_{50}s of II, III, IVd and Vd, which antagonized pentetrazole-induced convulsion, are shown in Table II. The ED_{50} of Vd was 10 times that of meprobamate.

Table IIIa. Effects of Maltol Analogs on the Convulsion induced by CNS Stimulants in Mice

Stimulant	Compound[a] Response																			
	Control					II					III					IVa				
	No.	CC	TE	D	S	No.	CC	TE	D	S	No.	CC	TE	D	S	No.	CC	TE	D	S
Pentetrazole (110 mg/kg *i.p.*)	20	19	19	18	1	10	8	9	9	1	10	4	3	3	6	10	3	1	1	1
Strychnine (1.8 mg/kg *i.p.*)	20	—	19	19	1	10	—	10	10	0	10	—	10	9	1	10	—	3	0	10
Caffeine (300 mg/kg *i.p.*)	20	18	19	19	1	10	6	10	10	0	10	5	9	9	0	10	9	8	9	0
Picrotoxin (6.5 mg/kg *s.c.*)	20	20	19	19	1	10	10	10	10	0	10	10	10	10	0	10	3	1	5	5
Bemegride 48 mg/kg *i.p.*)	16	15	16	16	0	8	8	8	8	0	8	8	8	7	1	8	5[b]	5[b]	5[b]	1
Pipradrol (140 mg/kg *i.p.*)	16	16	—	15	1	8	4	—	8	0	8	0	—	8	0	8	1	—	7	1
Sodium pyromeconate (700 mg/kg *i.p.*)	16	—	14	14	2	8	—	7	6	2	8	—	0	0	0	8	—	0	0	0

a) Dose of compounds: 3.96 mmol/kg *s.c.*
b) Retardation was observed.
CC: Clonic convulsion, TE: Tonic extension, D: Dead within 2 hr, S: Survival over 48 hr.

Table IIIb. Effects of Maltol Analogs on the Convulsion induced by CNS Stimulants in Mice

Stimulant	Compound[a] Response																			
	Control					II					IVd					Vd				
	No.	CC	TE	D	S	No.	CC	TE	D	S	No.	CC	TE	D	S	No.	CC	TE	D	S
Pentetrazole (110 mg/kg *i.p.*)	19	12	16	16	3	20	15	18	16	3	10	4	1	0	8	10	3	1	1	9
Strychnine (1.8 mg/kg *i.p.*)	20	—	17	16	4	20	—	17	17	3	9	—	5	2	7	10	—	9	1	7
Caffeine (300 mg/kg *i.p.*)	10	10	10	10	0	10	10	10	10	0	10	10	10	10	0	10	10	10	10	0
Picrotoxin (6.5 mg/kg *s.c.*)	20	19	19	19	1	20	16	15	18	2	10	10	1	3	4	10	10	1	3	5
Bemegride (48 mg/kg *i.p.*)	20	20	20	20	0	19	17	19	19	0	10	7	9	8	0	9	7	5	4	4
Pipradrol (140 mg/kg *i.p.*)	10	10	—	6	4	5	5	—	3	2	9	7	—	4	3	10	3	—	3	3

a) Dose of compounds: 3.96 mmol/kg *s.c.*
CC: Clonic convulsion, TE: Tonic extension, D: Dead within 2 hr, S: Survival over 48 hr.

2. Effect on Central Stimulant-Induced Convulsion

Table III shows the effects of some 2-alkylpyromeconic acids on the convulsions induced by pentetrazole, strychnine, caffeine, picrotoxin, bemegride, pipradrol and pyromeconate. Increase in the carbon number of the 2-alkyl substituent led to an increase in the inhibitory effects on the convulsions induced by pentetrazole, strychnine and picrotoxin. Compounds III, IVa and Vd each showed a depressing effect on the clonic convulsion induced by pipradrol but had no effect on lethality. None of the compounds listed in Table III showed anticonvulsant effect against caffeine. The effect of Vd on the convulsion induced by pentetrazole or picrotoxin was similar to that of IVd, whereas its effect on the convulsion induced by strychnine, bemegride or pipradrol was slightly different from that of IVd.

3. Effect on the Maximal Electroshock Seizure (MES)

The inhibitoy effects of II, III, IVb, IVd, Vb and Vd on the MES were studied (Table IV). A dose of 3.96 mmol/kg of II showed no inhibitory effect, whereas III, Vb or IVd had as the same effect as 200 mg/kg (0.92 mmol/kg) of meprobamate. In contrast to the effect on the pentetrazole-induced convulsion, Vb and Vd (which have a 1-hydroxyl group on the 2-alkyl substituent) showed smaller anticonvulsant effects on the MES than IVb and IVd, respectively.

Table IV. Effects of Maltol Analogs and Anticonvulsant Agents on Maximal Electroshock Seizure in Mice

Compound	Dose (mg/kg)	Dose (mmol/kg)	Route	Response[a] A/B
II	500	3.96	*s.c.*	10/10
III	556	3.96	*s.c.*	4/10
IVb	667	3.96	*s.c.*	2/10
Vb	730	3.96	*s.c.*	4/10
IVd	667	3.96	*s.c.*	3/10
Vd	730	3.96	*s.c.*	7/10
Phenytoin sodium	15	0.054	*i.p.*	0/10
Phenobarbital	15	0.065	*i.p.*	7/11
Meprobamate	200	0.916	*s.c.*	5/10

Stimulus intensity: 25 mA, 0.2 sec.
Mice were shocked 60 min after administration.
a) A: The number of animals which showed tonic extension.
B: The number of animals used.

Table V. Quantitation of the Effects of Maltol Analogs and Anticonvulsant Agents on the Maximal Electroshock Seizure in Mice

Compound	Route	No.	ED_{50} (mg/kg)	ED_{50} (mmol/kg)
II	*s.c.*	9	>2000	>15.9
III	*s.c.*	9	455	3.25
IVd	*s.c.*	9	643	3.82
Vd	*s.c.*	9	1760	9.56
Phenytoin sodium	*i.p.*	9	7.99	0.029
Phenobarbital	*i.p.*	9	13.4	0.058
Meprobamate	*s.c.*	9	241	1.10

Stimulus intensity: 25 mA, 0.2 sec.
Mice were shocked 30 min after dosing.

The ED_{50} of Vd for the MES (Table V) was higher than its LD_{50} (Table VI). The ED_{50} (mmol/kg) of III for the MES was lower than for pentetrazole-induced convulsion (Table II) and was about 3 times that of meprobamate.

4. Acute Toxicity

II, III, IVd and Vd showed almost the same toxicities (Table VI).

5. Effect on Spontaneous Motor Activity

As shown in Table VII, the spontaneous motor activity was depressed by about 50% after the administration of 1.07 mmol/kg of II, III, IVd or Vd. In spite of its very weak effect on the pentetrazole-induced convulsion, II showed fairly marked inhibition (66%) of the spontaneous motor activity.

Table VI. Acute Toxicity of Maltol Analogs in Mice

Compound	Route	LD_{50} (19/20 Confidence limits)	
		(mg/kg)	(mmol/kg)
II	*s.c.*	990 (856—1145)	7.85
III	*s.c.*	1200(1017—1416)	8.56
IVd	*s.c.*	1630(1463—1816)	9.69
Vd	*s.c.*	1510(1418—1608)	8.20

Table VII. Effects of Maltol Analogs and Central Depressants on Spontaneous Motor Activity in Mice

Compound	Dose[a] (mg/kg)	Route	No.	Total activity[b] (Counts/60 min)		Inhibitory percent
				Control	Compound	
Vehicle	—	*s.c.*	10	995± 88	870±108	—
II	135	*s.c.*	10	872±136	295± 31[c]	66.2
III	150	*s.c.*	9	699± 51	407± 78[d]	41.8
IVd	180	*s.c.*	8	748±102	266± 53[c]	64.4
Vd	197	*s.c.*	8	617± 80	302± 64[e]	51.2
Meprobamate	70	*s.c.*	12	812±101	519± 62[c]	36.1
Chlorpromazine	5	*i.p.*	10	630± 82	92± 34[e]	85.4

a) Doses of maltol analogs were 1.07 mmol/kg.
b) Counts are expressed as means±s.e.
c) $p<0.01$.
d) $p<0.02$.
e) $p<0.001$.

Table VIII. Effects of Maltol Analogs on Hexobarbital-Induced Sleeping Time in Mice

Compound	Dose[a] (mg/kg)	Route	No.	Sleeping time (min)		Prolongation rate (control=1)
				Control	Compound	
II	135	*s.c.*	9	53.6±7.1	66.2± 7.7	1.24
III	150	*s.c.*	10	56.5±3.1	93.0±12.7[b]	1.65
IVd	180	*s.c.*	10	62.6±8.0	65.9± 5.2	1.05
Vd	197	*s.c.*	10	64.4±7.6	119.5±11.5[c]	1.86

Dose of hexobarbital sodium: 100 mg/kg *i.p.*
Data are expressed as means±s.e.
a) 1.07 mmol/kg.
b) $p<0.02$.
c) $p<0.001$.

6. Effect on the Hexobarbital-Induced Sleeping Time

Neither II nor IVd (1.07 mmol/kg) prolonged the hexobarbital-induced sleeping time (Table VIII). About 1.9-fold prolongation of the sleeping time was caused by Vd.

7. Muscle Relaxation Effect

Table IX shows the muscle relaxation effects of II, III, Vb and Vd; III was the strongest among them.

8. Solubility and Partition Coefficient

The solubilities and the partition coefficients of II, III, IVd and Vd are shown in Tables X and XI. The solubility in organic solvents and the partition coefficient increased with increasing carbon number of the 2-alkyl substituent. The solubility of Vd, a more polar compound, was lower in organic solvents and higher in water than that of IVd. The partition coefficient of Vd was very small.

9. Tissue Level

Table XII shows the levels of II, III, IVd and Vd in plasma, brain and liver after the subcutaneous administration of 3.96 mmol/kg. The plasma level of II or III was almost the same at 30 and 60 min after dosing, while that of IVd or Vd at 60 min after dosing was higher than that at 30 min. The plasma and brain levels of IVd were low.

Table IX. Effects of Maltol Analogs on Muscle Relaxation in Mice

Compound	Route	ED_{50} (19/20 Confidence limits)	
		(mg/kg)	(mmol/kg)
II	*s.c.*	762(693—838)	6.04
III	*s.c.*	458(405—517)	3.27
Vb	*s.c.*	680(576—802)	3.69
Vd	*s.c.*	770(695—855)	4.18

Table X. Solubilities of Maltol Analogs

Solvent	Solubility[a]			
	II	III	IVd	Vd
Heptane	30000	5700	340	Insoluble
Petroleum Ether	15500	4000	170	Insoluble
Benzene	170	10	3	2200
Chloroform	20	1.75	2	100
Ethanol	70	7	2.5	15
Water	220	95	600	150

a) The solubility is expressed as the minimum volume (ml) of the solvent required to dissolve 1 g of the compound.

Table XI. Partition Coefficients for Maltol Analogs

Solvent	Organic Solvent/Buffer (pH 7.4) distribution ratio			
	II	III	IVd	Vd
Heptane	0.009±0.001	0.063±0.009	1.39±0.02	—
Benzene	0.431±0.006	2.04 ±0.01	20.9 ±0.9	0.048±0.008
Chloroform	3.91 ±0.02	20.5 ±0.6	111.6 ±7.3	0.697±0.009

Values are the means of three experiments±s.e.

Table XII. Tissue Levels of Maltol Analogs after Subcutaneous Administration in Mice

Compound	Dose[a] (mg/kg)	min[b]	Concentration (mean ± s.e.) Plasma μg/ml	Plasma μ mol/g	Brain μg/g	Brain μ mol/g	Brain/Plasma	Liver μg/g	Liver μ mol/g	Liver/Plasma
II	500	30	261 ±32(4)	2.07	213 ±17(5)	1.69	0.82	229 ±21(5)	1.82	0.88
		60	251 ±17(4)	1.99	216 ± 5(4)	1.71	0.86	233 ±43(4)	1.85	0.93
III	556	30	297 ±34(5)	2.12	224 ±13(5)	1.60	0.75	255 ± 6(4)	1.82	0.86
		60	317 ±15(5)	2.26	250 ± 5(4)	1.78	0.79	356 ± 6(5)	2.54	1.12
IVd	667	30	44.9±28.0(5)	0.267	46.7± 1.4(4)	0.278	1.04	88.8± 6.8(5)	0.528	1.98
		60	78.6±11.5(5)	0.467	39.7± 5.2(5)	0.236	0.51	107 ±14(4)	0.636	1.36
Vd	730	30	322 ±35(5)	1.75	285 ±13(5)	1.55	0.89	309 ±30(5)	1.68	0.96
		60	445 ±18(5)	2.42	350 ±11(5)	1.90	0.80	357 ±21(5)	1.94	0.79

Numbers in parentheses represent the numbers of experiments.
a) 3.96 mmol/kg.
b) The time after dosing.

Discussion

The relationship between the inhibitory effect of the analogs of II on pentetrazole-induced convulsive death and their chemical structures can be summarized as follows:

1) The effect increased with lengthening of the carbon chain of the 2-substituent of I, and that of the 2-butyl compound was the strongest.

2) Introduction of a hydroxyl group into the 2-alkyl substituent increased the effect.

3) The effect was not increased by substitution of a methoxyl for the hydroxyl group at the 3-position of the 4-pyrone ring.

4) Replacement of the oxygen atom in the ring with an =NH group did not alter the effect.

5) No difference was found between the effect of II, which has a 2-methyl group, and that of X or VIII which has a 6-methyl or a 6-hydroxymethyl group, respectively.

6) I showed a weak effect ($p<0.05$), though it is known to cause convulsion at higher doses.[14]

The effects of several 2-alkylpyromeconic acids on central stimulant-induced convulsion were as follows:

1) The inhibitory effect on the pentetrazole-, strychnine- and picrotoxin-induced convulsions increased with increasing carbon number of the 2-alkyl group.

2) None of the compounds tested showed anticonvulsant effect against caffeine.

3) Vd showed anticonvulsant effect against bemegride, and IVa showed slight activity.

4) III and IVa depressed the tonic extension due to sodium pyromeconate.

The relationship between the length of the carbon chain in the 2-alkyl substituent and the inhibitory effect on pentetrazole-induced convulsion was not seen in the case of the MES. The effect of a compound having a 2-(1-hydroxy)alkyl substituent was rather weaker than that of a compound having the corresponding 2-alkyl substituent, and the ED_{50} of III, the 2-ethyl compound, was the lowest among the compounds tested. The anticonvulsant effects of II analogs tested were observed at high doses near to the LD_{50}s. Thus, the results of the present study suggest that the II analogs tested were not potent anticonvulsants.

There was no correlation between the carbon number of the 2-alkyl substituent and the extent of depressing effect on spontaneous motor activity or of prolonging effect on the hexobarbital-induced sleeping time. Namely, 1.07 mmol/kg of II, III, IVd or Vd depressed the

14) M. Takemura, Y. Inazu, and H. Takagi, *Takamine Kenkyusho Nempo*, **5**, 114 (1953).

spontaneous motor activity significantly, and the potencies of II and IVd were similar. The same molar doses of III and Vd prolonged the hexobarbital-induced sleeping time significantly, while such doses of II and IVd did not. (However, II showed a prolonging effect at a dose of 300 mg (2.38 mmol)/kg *s.c.*, as described in the previous paper.[1])

The ED_{50} for the muscle relaxation effect of II was about 3/4 of its LD_{50}, while that of III was less than half of its LD_{50}. The ED_{50}s of II, III and Vd for muscle relaxation did not correlate with their ED_{50}s for the pentetrazole-induced convulsion and the MES. It appears that their muscle relaxant effects might be independent of their anticonvulsant actions.

It is generally accepted that the lipid solubility of a drug is an important factor in connection with its transfer into the central spinal fluid and brain. The increase of inhibitory effect of 2-alkylpyromeconic acids on the pentetrazole-induced convulsion with increasing carbon number of the 2-alkyl group might be due to enhancement of lipid solubility. Thus, the solubilities in organic solvents or water, the partition coefficients and the tissue levels of II, III, IVd and Vd after administration were measured. The lipophilicity of IVd was the highest and its water solubility was the lowest among them. The lipophilicity of Vd was the lowest, and its water solubility was higher than that of IVd, but lower than that of III. No correlation could be seen between the lipid solubilities and the concentrations in the plasma or brain; the levels of IVd in particular were thought rather to reflect its low solubility in water. The brain level of II, III or Vd was almost the same as the plasma level. This result indicates high permeability from the blood to the brain. Taking account of the brain level, the anticonvulsant and spontaneous motor activity-depressing effects of IVd appear to be the most potent among those of the drugs tested.

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