Summary

NS 2662, DMVP, trichlorfon and dichlorvos were applied to the American cockroach and the mouse, and the change in free amino acids contents in the cockroach and the mouse brain were investigated.

1) As free amino acids in the cockroach whole body, 17 amino acids including proline, glutamine, glutamate and alanine, and two ninhydrin positive substances were detected. Besides the above amino acids, GABA was detected in the cockroach nerve tissue. In the mouse brain, besides above 17 amino acids, GABA, taurine and one ninhydrin positive substance were detected.

2) Proline and alanine contents in the cockroach whole body decreased with the process of poisoning. At the convulsive and paralyzed stages, glutamine content increased and glutamate content decreased. In the nerve tissue of paralyzed cockroach, GABA, glutamate and alanine contents decreased.

3) In the brain of the mouse poisoned with the insecticides, glutamine content increased, but glutamate was almost the same as in the brain of the untreated mouse. Alanine content increased temporarily and decreased. A little decreasing of GABA content was observed in the brain of NS 2662 poisoned mouse and it was increased in the brain of the mouse poisoned with other insecticides.

Residues of the Acaricide Proclonol in Fruits and Animal Tissues. Toshiie NAKAMURA, Mitsuru ANDO, Harue TAMARI, Eiko MATSUBAYASHI, and Mitsuru UCHIYAMA* (Agricultural Chemicals Research Laboratories, Sankyo Co., Ltd., Shinagawa-ku, Tokyo, and *Pharmaceutical Institute, Tohoku University, Aobayama, Sendai) Received March 20, 1973. *Botyu-Kagaku*, 38, 99, 1973.

15. 殺ダニ剤 Proclonol の果実および動物体残留性 中村利家，安藤満，玉利春江，松林英子，内山充* （三共株式会社農薬研究所，東京都品川区広町1-2-58，*東北大学薬学部，仙台市青葉山980）
48. 3. 20 受理

殺ダニ剤proclonol〔キラカール®，di-(*p*-chlorophenyl)-cyclopropyl carbinol〕の残留分析法を確立し，果実と動物組織中の残留蓄積量を測定した．Proclonol は ECD 付ガスクロマト法により最少限 1ng の検出が可能であり，アセトン抽出後のクリーンアップはヘキサン—DMSO 分配，フロリジルカラムクロマト，およびけん化の組合せでおこなった．果実ではミカン，リンゴおよびナシの残留量を測定したが，ミカンの果肉部へのしん透残留は全く認められず，果皮表層ワックス中に固定されていると思われた．動物体の場合 proclonol を3カ月間連続投与したラットとマウスの組織を分析したが，蓄積性は DDT で知られているレベルよりはるかに低く，1/6またはそれ以下と考えられる結果であった．

Proclonol〔di-(*p*-chlorophenyl)-cyclopropyl carbinol, Kilakar®〕is a potent acaricide which has been used for the control of the citrus mites in this country. It is characterized by advantage of its compatibility with alkaline pesticides such as Bordeaux Mixture and lime sulfur, since it does not contain unstable group such as trichloromethyl group in dicofol. Its mammalian toxicity is relatively low; the acute oral LD_{50} to mice was 3420mg/kg, and the non-effect levels to rats and mice in feeding tests for 90 days were about 25 and 12mg/kg/day, respectively[1].

This work was undertaken to determine the residues of proclonol in plants and animal tissues.

Materials and Methods

Sample materials

Fruits : A sufficient amount of 40% wettable powder formulation (WP) or 40% emulsifiable concentrate (EC) diluted to 1500-fold with water was sprayed 2 to 4 times to the fruit plants in the fields and fruits were collected from the prefectural agricultural experimental stations as shown in Table 2. The samples were kept in a freezer at −20°C until the analyses were started.

Animal tissues : Sprague-Dawley strain rats

weighing ca. 110g (female) and 130g (male) were fed on diets containing proclonol at concentrations of 1000 to 4000 ppm for 90 days in the Central Research Laboratory, Sankyo Co. One group to each dosage and sex consisted of 10 animals. Soon after sacrifice of the rats, the brain, liver, kidney, and intraperitoneal fat tissues were dissected out and grouped by each dose level and sex. The tissues were weighed and kept in a freezer at −20°C. Wistar strain rats weighing ca. 130g (female) and 150g (male) and DDY strain mice weighing ca. 22.5g (female) and 25g (male) were fed at dietary concentrations of 62.5 to 1000 ppm for 90 days in the pharmaceutical Institute, Tohoku University. One group to each dosage and sex consisted of 5 animals. Soon after sacrifice, the liver and intraperitoneal fat tissues were dissected out from each animal and weighed. The tissues were individually immersed in acetone and kept in a refrigerator at −5°C.

Apparatus and reagents

Warring blender : Universal homogenizer with 500m*l* cup (NIHON SEIKI SEISAKUSHO Co.). Shaker : V-D type KM Shaker (IWAKI Co.). Acetone and *n*-hexane : redistilled. Diethylether, dimethylsulfoxide (DMSO), benzene, methanol, anhyd. sodium sulfate, and KOH : reagent grade. Florisil (FLORIDIN Co.) : activated at 130°C for 4 hr and stored in a desiccator. Proclonol : recrystallized from hexane, mp 63°C. Phenkapton, *O*, *O*-diethyl-*S*-(2, 5-dichlorophenylthio)-methyl phosphorodithioate (internal standard in gas chromatography) : analytical grade standard.

Extraction

Fruits : The chopped samples (fruits 100g, orange peel 20g) were put into a blender cup with 80m*l* of acetone, and homogenized for ca. 3 min. The homogenized solution was centrifuged at 3000 rpm for 10 min and the supernatant was transferred to a 300m*l* separatory funnel. Acetone (70m*l*) was added to the residue and same procedure was repeated. The supernatant extracts were combined.

Animal tissues : The chopped samples (liver 5 to 10g, kidney, fat and brain 1 to 2g) were blended with 60m*l* of acetone for 15 min and the extracts were separated by centrifugation for liver or filtration with a glass wool plug for others. The residues were extracted with 60m*l* of acetone by the same procedures described above. The extracts were combined.

Cleanup procedures

Transfer to hexane : To each acetone extract, 100m*l* of 2% aq. solution of sodium sulfate and 25m*l* of hexane were added. After shaking for 10 min, the hexane layer was separated and dried over anhyd. sodium sulfate.

Partitioning : The hexane layer was transferred to a 100m*l* separatory funnel, and 30m*l* of DMSO saturated with hexane was added. After shaking for 10 min, the DMSO layer was separated and transferred to a 200m*l* separatory funnel, and 80m*l* of 2% aq. solution of sodium sulfate and 25m*l* of hexane were added. After shaking for 10 min, the hexane layer was separated and dried over anhyd. sodium sulfate.

Florisil column chromatography : A chromatographic column (15mm i.d., 30cm long) was plugged with glass wool, and 5g of the activated Florisil was poured into the tube as a slurry with hexane. When hexane had drained away, anhydrous sodium sulfate was charged on the surface at the depth of ca. 15mm. The hexane extract was applied to the column and eluted with 150m*l* of 15% ether in hexane at the rate of 5 to 10m*l*/min. The eluate was evaporated *in vacuo* to just dryness at 40°C with a rotary evaporator.

Saponification : To the residue, 5m*l* of N/2 methanolic KOH solution was added and refluxed for 30 min at 80°C with an air condenser. The solution was transferred to a 200m*l* separatory funnel with 80m*l* of 2% aq. solution of sodium sulfate and 25m*l* of hexane. After shaking for 10 min, the hexane layer was separated and dried over anhyd. sodium sulfate.

Gas chromatography

Apparatus : Gas chromatograph : F & M model 402 equipped with ECD (^{3}H). Gas chromatographic column : U-shaped glass column (3mm i.d., 135cm long) containing ca. 2% silicone XE-60 on 80/100 mesh chromosorb W(AW, HMDS) which was prepared by the infiltration technique.

Operating conditions : Gas flows : carrier gas (He) 80 to 100 m*l*/min, purge gas (Argon

containing 10% methane) 140 ml/min. Temperatures: column 180 to 185°C, injection port 190 to 195°C, detector 200 to 210° C.

Calibration and determination: The amount of proclonol in each sample was determined by comparing the peak height of proclonol with that of the internal standard and by reference to a calibration curve prepared by plotting weight ratios against peak height ratios of proclonol to the internal standard.

Results and Discussion

Gas chromatography

The electron capture detection was sensitive to proclonol and 1ng was the minimum detectable amount in these conditions. Phenkapton was mainly used for the internal standard substance, since the retention time relative to proclonol was appropriate (ca. 1.7) and separation from the interfering peaks due to biological materials was satisfactory. Chloropropylate, binapacryl (Acricid), dioctylphthalate (DOP), and EPN were also adaptable properly as the internal standard. Relative t_R were ca. 0.8, 1.3, 1.3 and 3.1, respectively.

Typical chromatograms and calibration curves are shown in Fig. 1 and 2 respectively.

Extraction and cleanup

As the homogenizing solvent for the extraction, acetone was chosen in order to avoid the emulsification problems.[2,7] Proclonol in the extracts was almost completely partitioned into hexane in the presence of 2% aq. solution of sodium sulfate. Further cleanup was accomplished by combination of the partitioning,[3,4] the Florisil column chromatography,[5,6] and the saponification[4].

In the partitioning for separation of fats, waxes and pigments, the partition coefficient of proclonol was greater in DMSO/hexane (ca. 22) than in acetonitrile/hexane (ca. 5. 4). In the Florisil column chromatography, proclonol was thoroughly eluted by first 100ml of ether/hexane (15/85), but 150ml of the eluting mixture was employed for analysis in the biological samples. As the final cleanup procedure for removing the interfering peaks near proclonol on gas chromatograms, the saponification with methanolic KOH was conducted. Proclonol was stable under refluxing in N/2 methanolic KOH for 30 min, since it does not contain any alkaline sensitive group.

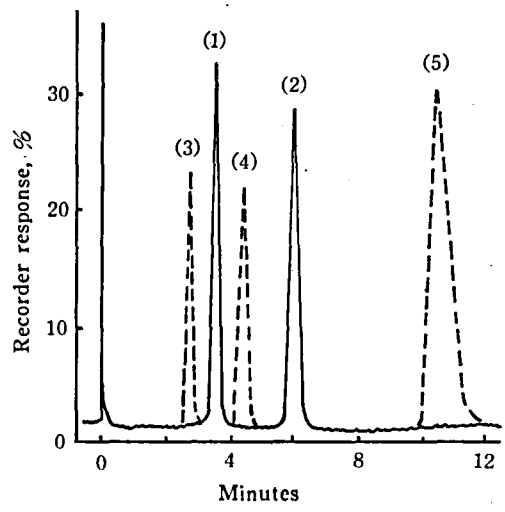

Fig. 1. Typical chromatograms of proclonol and internal standards.
(1) Proclonol (10ng) (2) Phenkapton (0.5ng) (3) Chloropropylate (ca. 5ng) (4) Dioctylphthalate (ca. 2.5ng) and Binapacryl (ca. 0.5ng) (5) EPN (ca. 0.6ng)

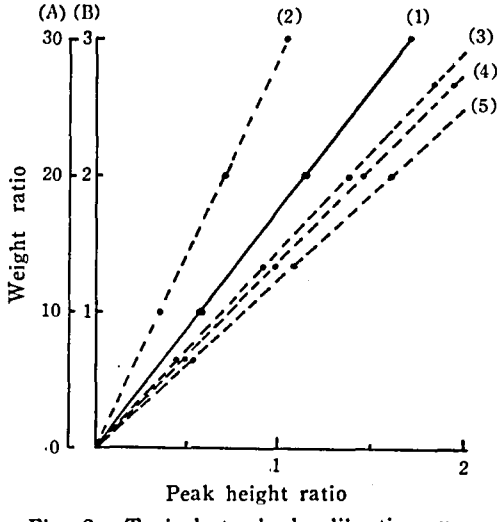

Fig. 2. Typical standard calibration curves. Internal standard: (1) Phenkapton (A), (2) Dioctylphthalate (B), (3) EPN (A), (4) Chloropropylate (B), (5) Binapacryl (A) (A) and (B) show the distinction in the scale of weight ratio.

Table 1. Recoveries of Proclonol from Fortified Samples

Sample	Proclonol added	Replication	Recovery	
			Av.	Range
Fruits				
Orange pulps	0.1～0.6ppm	6	87%	78～95%
Orange peels	0.5～1.0	2	96	95～97
Apples	0.1～0.5	2	84	82～86
Pears	0.1	2	85	79～90
Rat tissues				
Brains	1.0	2	81	79～83
Livers	1.0	2	101	99～103
Kidneys	1.0	2	88	78～103
Intraperitoneal fat	1.0	1	93	—

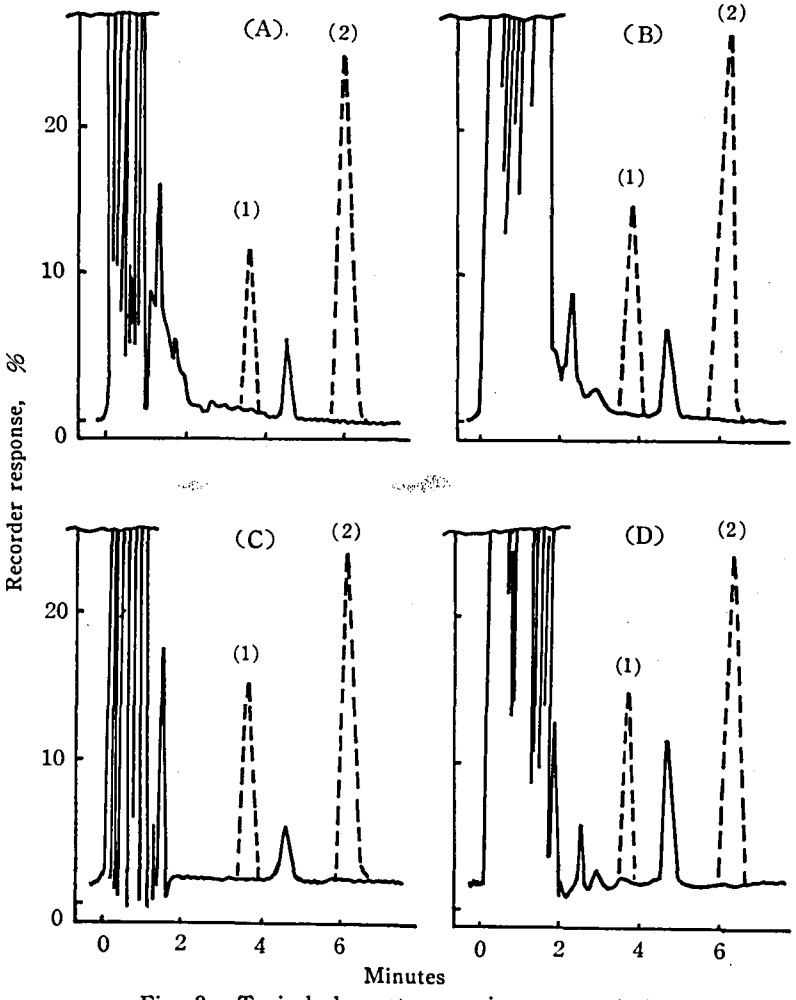

Fig. 3. Typical chromatograms in recovery tests.
(A) Orange pulp (B) Orange peel
(C) Rat liver (D) Rat body fat
(1) Proclonol
(2) Phenkapton (Internal standard)

The cleanup in low-waxy fruit samples such as orange pulps, apples and pears was sufficiently conducted with Florisil column chromatography and saponification. However, in waxy or oily materials such as orange peels and animal tissues, the partitioning was necessary at the first step in the cleanup.

The overall recoveries to the fortified samples are summarized in Table 1. Recoveries of proclonol fortified at levels of 0.1 to 1.0 ppm were over 80% in most of the cases. The result indicates that the proposed methods are feasible for the residue determination of proclonol. The minimum detectable level was approximately 0.02 ppm in fruits, 0.1 ppm in orange peels and 0.2 ppm in animal tissues, although they are variable by sampling scales.

Some typical gas chromatograms in recovery tests are shown in Fig. 3.

Residue levels in fruits

Residue levels of proclonol in oranges, apples, and pears are summarized in Table 2.

The residue in orange pulps was not detectable but that in orange peels was found within the range of 1 to 7 ppm. It means that proclonol is deposited in the waxy or oily peels and permeates scarcely into the pulp portion. It has been indicated by Gunther[19] that chlorobenzilate does

Table 2. Residues of proclonol on and in Fruits

Fruit	Formulation	Times of applications	Proclonol found[a] Days[b]	ppm	Days[b]	ppm
			Sampling place			
			KANAGAWA		TOKUSHIMA	
		2	30	<0.02	34	<0.02
	WP		51	<0.02	—	
		4	17	<0.02	15	<0.02
			38	<0.02	30	<0.02
Orange pulps			OSAKA		MIYAZAKI	
		2	14	<0.02	15	<0.02
	EC		30	<0.02	30	<0.02
		4	14	<0.02	15	<0.02
			30	<0.02	30	<0.02
			KANAGAWA		TOKUSHIMA	
		2	30	2.35	34	2.04
Orange peels	WP		51	0.90	—	
		4	17	4.25	15	7.08
			38	5.56	30	6.50
			AOMORI		IWATE	
		2	7	0.49	30	0.60
Apples	WP		14	0.46	45	0.55
		3	30	0.49	30	0.63
			44	0.50	45	0.60
			NAGANO		TOTTORI	
		2	30	0.02	30	<0.02
Pears	WP		41	<0.02	—	
		3	10	0.06	10	0.03
			21	0.05	20	0.02

a) Average of duplicate determinations.
b) Days after the final application.

not penetrate into the juice or pulp portions of lemon. The residue level in apples ranged from 0.46 to 0.63 ppm. The decreasing rate of the residue in apples was not always remarkable, since the growing rates of fruits in this experiment periods were also not significant. Proclonol may have deposited stably in the surface waxy layer. The residue level in pears was negligible, since the fruits were covered by paper bags throughout the growing period.

The permissible level(PL) of proclonol in fruits could be calculated by WHO/FAO rule[8]and the provided rule in this country as follows :

$$PL=\frac{\text{Acceptable daily intake (ADI)} \times \text{Average body weight}}{\text{Food factor}}=2.22 \text{ ppm}$$

$$ADI=\frac{\text{Non-effect level (=12mg/kg/day in mouse)}^{1)}}{\text{Safety coefficient (=2000)}}=0.006 \text{ mg/kg/day}$$

Avarage body weight=50kg

Food factor on fruits=0.135kg/day

The residue levels in orange pulps, apples and pears were much lower than the permissible level.

Storage in animal tissues

First, the storage in rats (Sprague-Dawley strain) fed on the extremely high dietary concentration (1000~4000 ppm) was determined in order to know the distribution of proclonol. As shown in Table 3, the storage in fatty tissues was greater than that in non-fatty tissues, as well-known for other lipophilic pesticides.

In the case of rats (Wister strain) fed on dietary concetrations of 62.5 to 1000 ppm, the storage amounts were determined in the intraperitoneal fat and liver tissus. The results are shown in Table 4. It is noteworthy that the storage levels in the fat of female rats are coincidental to the dietary concentrations of proclonol. In male rats, the storages in the fat were approximately one-fourth of those in female. The storage levels in the liver were remarkably lower than those in the intraperitoneal fat.

Table 3. Distribution of proclonol in some Tissues of Sprague-Dowley Rats after the 90 days Feeding Test at Dietary Concentrations of 1000 to 4000 ppm.

Tissue	Proclonol conc., ppm to fresh tissue[a)]					
	Dietary conc., ppm					
	1000		2000		4000	
	Female	Male	Female	Male	Female	Male
Brains	8.1	1.6	18.9	8.1	38.5	38.9
Kidneys	10.9	2.5	43.8	15.4	74.1	65.0
Livers	16.1	5.5	41.9	19.1	94.4	100.4
Body fats	672	178	1672	1078	—	—

a) Average of duplicate determinations

Table 4. Storage of Proclonol in Intraperitoneal Fats and Livers of Wistar Rats after the 90 days Feeding Tests at Dietary Concentrations of 62.5 to 1000 ppm.

Dietary conc.	Proclonol conc., ppm to fresh tissue[a)]			
	Body fats		Livers	
ppm	Female	Male	Female	Male
62.5	57.2±16	13.9± 4.7	2.1±0.7	1.0±0.4
125	100 ±51	22.3± 5.2	3.3±1.6	1.7±0.4
250	270 ±92	70.8±37	7.7±2.5	3.8±1.1
500	490 ±30	121 ±34	12.4±2.4	5.7±2.4
1000	945	216	22.0	8.2

a) Average of 5 rats at dietary concentrations from 62.5 to 500 ppm and 2 rats at 1000 ppm. The storage was determined for each animal in this experiment.

Table 5. Storage of Proclonol in Body Fats and Livers of Mice after the 90 Days Feeding Test at the Dietary concentrations of 62.5 to 1000 ppm.

Dietary conc., ppm	Proclonol conc., ppm to fresh tissue[a]			
	Body fats		Livers	
	Female	Male	Female	Male
62.5	33.3	49.4	7.0	7.9
125	81.1	117	6.7	10.5
250	356	456	18.6	30.2
500	496	481	19.5	35.7
1000	492	659	39.4	45.0

[a] Average of duplicate determinations.

However, the storages in male were approximately a half of those in female. It has been known that the sex difference in the storage of pesticides is remarkable in rats[12,15] but not significant in other animals such as dog and monkey.[15]

The storage levels in the intraperitoneal fat and liver tissues of mice are shown in Table 5. Tendency of the storage was roughly similar to that in the case of rats; however, the sex difference was not noticeable: The storage in female was rather lower than that in male. The storage amount-dietary concentration curves came near plateau in the 250 ppm dietary concentration, which was equivalent to the certain toxic concentration level[1]. There are many reports on the storage in rats of DDT[9-16] and dicofol[17,18]. These storage data in the fat under the similar experimental conditions indicate that the storage levels of DDT are approximately 10 to 20 times to the dietary concentration in female rats and 5 to 10 times in male rats, and that of dicofol are approximately 1.5 times in female rats and one-hlaf in male rats.

It may be considered that the storage level of proclonol in animals will be remarkably lower when compared with DDT and nearly equal to dicofol. The presence of hydrophilic OH group in both molecules may be a function to control the storage.

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