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Glycerol and related compounds as feeding stimulants for cockroaches

By

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INTRODUCTION

Among several sugary substances, sorbitol, and mannitol, as well as several sugars, have shown a particular feeding-stimulative effect on the German cockroach, *Blattella germanica* (Tsuji, 1965). Sorbitol was effective also for other two species, *Periplaneta americana* and *P. fuliginosa*, whereas mannitol was not effective for *P. fuliginosa* but somewhat effective for *P. americana*. Dulcitol and meso-inositol were not or less effective for the three species. The results of these polyols (Tsuji, 1965) suggest a possibility that some of more simple polyols might also be feeding-stimulative for the cockroaches. The present study was designed to examine the feeding responses of the cockroaches to several glycols, glycerol and related substances.

MATERIALS AND METHODS

Test insects: The cockroaches used in the filter paper method were *Periplaneta americana* Linné, *P. fuliginosa* Serville, and *Blattella germanica* (Linné), and in the bait method *B. germanica* alone. These had been reared in the laboratory throughout

the year by the same method as described in one of previous papers (Tsuji, 1965).

Filter paper method: The technique employed for the filter paper method was essentially the same as described in the previous paper (Tsuji, 1965). The water, acetone, or chloroform solutions of test compounds were dropped with pipets on a filter paper (12 cm in diameter), making spots of about 2 cm diameter (about 0.025 ml per spot). After being dried, the paper was placed in the test container of starved adult cockroaches and was allowed to remain overnight. In the cases of *P. americana* and *P. fuliginosa*, 10 cockroaches consisting of 5 male and 5 female adults were used in each of test containers, and in *B. germanica* 30 female adults were used in each. The cockroaches were supplied with enough water during the experiments. Bitten spots were easily recognized by tufts of filter paper.

Bait method: Using *B. germanica*, the effect of established feeding stimulants, glycerol, maltose, and sucrose was also evaluated by a bait method. The baits were compounded as tablets, each of which was about 1.8 g in weight, 2 cm in diameter, and 7 mm in thickness. The compositions of the tablets will be given in the next section. A detailed description of the method has been given by Tsuji and Ono (1969).

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Ingredients: The ingredients used were of the following sources, and purities or designated grades, which were expressed in the results (Tables 1, 2 and 3) by each of the following heading numbers and alphabetic letters.

Sources: 1) Sigma Chemical Co., St. Louis, 2) Applied Science Laboratories Inc. State College, Pennsylvania, 3) Tokyo Kasei Kogyo Co. Ltd., Tokyo, 4) Iwai Kagaku Co. Ltd., Tokyo, 5) Synthesized in our laboratory.

Purities or grades: a) Designated as more than 99% and were homogeneous when determined by thin layer chromatography. b) Purified in our laboratory by silica gel column chromatography until they were homogeneous as determined by thin layer chromatography. c) Designated as the special grade. d) Designated as the first grade.

Other ingredients such as starch, maltose, etc. were of the same sources and designated grades as used in the previous report (Tsuji and Ono, 1969).

RESULTS

I. Results obtained by the filter paper method.

Several glycols, glycerol, and some other polyols.

Ten or thirteen compounds of the same concentration were tested at a time on each filter paper. For each dose six repetitions (3 containers $\times$ 2 repetitions) were made. The results obtained in *P. americana* are shown in Table 1. Glycerol was strongly effective in eliciting the feeding response. Erythritol, D-arabitol and L-arabitol were moderately effective, whereas all glycols tested were not.

The results obtained in *P. fuliginosa* were essentially the same as obtained in *P. americana*. *B. germanica*, however, did not respond to erythritol, D-arabitol and L-arabitol even at the dose of 2.5 mg per spot.

Monoglycerides.

In the same way as in the test of the polyols, seven monoglycerides were tested. The results obtained in *P. americana* are shown in Table 2. None of the monoglycerides having C_4 — C_{18} saturated fatty acids as acid residues was effective, though some of the free fatty acids had been shown to be feeding-stimulative and/or attractive to the cockroaches (Tsuji, 1966). Monoolein with an unsaturated C_{18} fatty acid, on the other hand, was clearly feeding-stimulative. Monoacetin (with C_2 fatty acid) was some-

Table 1. Feeding response of *P. americana* to spots treated with simple polyols.

Compound	Source and grade	Dose per spot		
		25 mg	2.5 mg	0.25 mg
Ethylene glycol	3-c	-----	-----	-----
1,2-Propylene glycol	3-c	-----	-----	-----
1,3-Propylene glycol	3-d	-----	-----	-----
1,3-Butylene glycol	3-c	-----	-----	-----
1,4-Butylene glycol	3-d	-----	-----	-----
2,3-Butylene glycol	3-d	-----	-----	-----
1,5-Pentanediol	3-d	-----	-----	-----
2-Methyl-2,4-pentanediol	3-d	-----	-----	-----
2-Ethyl-1,3-hexanediol	3-d	-----	-----	-----
Glycerol	4-a	++++++	++++++	++++++
meso-Erythritol	3-c		++++++*	-----
D-Arabitol	3-c		+++--*	-----
L-Arabitol	3-c		++++--*	-----

One plus sign (+) expresses one spot responded by the cockroaches.

* *B. germanica* did not respond to the spots.

Table 2. Feeding response of *P. americana* to spots treated with monoglycerides.

Compound	Source and grade	Dose per spot	
		1.25 mg	0.25 mg
Monoacetin*	3-b	++----	+-----
Monocaproin*	5-b	-----	-----
1-Monocaprin	5-b	-----	-----
1-Monolaurin	3-b	-----	-----
1-Monomyristin	5-b	-----	-----
Monopalmitin*	3-b	-----	-----
1-Monostearin	3-b	-----	-----
1-Monoolein	3-b	+++++-	+++++-
Check (Glycerol)	4-a	++++++	++++++

* Presumably the major component was 1-monoglyceride.

what effective, but much less than glycerol and monoolein.

The results obtained in *P. fuliginosa* and *B. germanica* were similar to those in *P. americana*.

Triglycerides.

Ten triglycerides were tested in the same way as described above. The results obtained in *P. americana* are shown in Table 3. Tricaprin (with C₁₀ saturated fatty acid) was clearly effective, but triglycerides with C₂—C₈ and C₁₂—C₁₈ saturated fatty acids were not or less effective. While triolein and trilinolein, with C₁₈ unsaturated fatty acids, were effective.

The results obtained in the other two species were also similar to those shown in

Table 3. Feeding response of *P. americana* to spots treated with triglycerides.

Compound	Source and grade	Dose per spot	
		1.25 mg	0.25 mg
Triacetin	3-c	-----	-----
Tributylin	3-c	-----	-----
Tricaprylin	2-a	+-----*	+-----
Tricaprin	2-a	+++++-	+++++-
Trilaurin	3-b	+++++-	+++++-
Trimyristin	3-b	-----	-----
Tripalmitin	3-c	-----	-----
Tristearin	3-c	-----	-----
Triolein	3-b	+++++-	+++++-
Trilinolein	1-a	+++++-	+++++-
Check (Glycerol)	4-a	++++++	++++++

* *B. germanica* responded more (++++--).

Table 3.

Comparison between glycerol and maltose.

Maltose has been shown to be the strongest feeding stimulant sugar for the three species (Tsuji, 1965; Tsuji and Ono, 1969). So, comparisons were made between maltose and glycerol with the three species. On each filter paper, eight spots consisting of four spots of each compound were made. As a rule, the two compounds were compared in the same molar dose on each filter paper, but different molar doses were also compared in *B. germanica*. The results are shown in Table 4.

The results obtained in *P. americana* and

Table 4. Comparison between glycerol and maltose.

Molar concentration of maltose or glycerol in solution, 25 μ l of which was given to each spot.		1/10 M.		1/20 M.		1/40 M.		1/80 M.	
Dose of maltose or glycerol per spot (mg)		0.90	0.23	0.45	0.12	0.23	0.06	0.11	0.03
Species	Compound*	M	G	M	G	M	G	M	G
<i>P. americana</i>	(5♀)	+++--	+++--	+++--	+++--	+++--	+++--	-----	-----
<i>P. fuliginosa</i>	(5♀)	+++++	+++++	+++++	+++++	+++++	+++++	-----	-----
<i>B. germanica</i>	(30♀)	+++++	+++++	+++++	-----	-----	+++++	-----	-----
					-----	+++++			
					-----			+++--	

* M: Maltose, G: Glycerol.

P. fuliginosa show that glycerol was as effective as maltose with respect to the threshold molar dose, and naturally much more effective than maltose with respect to the threshold weight dose. For *B. germanica*, however, glycerol was slightly less effective than maltose in regard to both the threshold molar and weight doses.

II. Results obtained by the poison bait method.

Effects of glycerol.

The effect of the addition of glycerol to practical poison baits was evaluated with *B.*

germanica.

In the first test, eight tablets each of which contained 0, 1, 2, 4, or 8% glycerol in weight were tested in each test container with 30 female adults. The tablets were exposed to the insects for 2 days. The compositions of the tablets and the results of three repetitions (three containers) are shown in Table 5. In the second test, which was similar to the first, tablets were exposed to the insects for 3 days. The results are shown in Table 6. In the third test, only three tablets were used in each container. The tablets were exposed to the in-

Table 5. Effect of glycerol in practical poison baits (I).

Weight composition of tablets (%)	Glycerol	0	1	2	4	8
	Boric acid	20	20	20	20	20
	Methylmyristate	0.2	0.2	0.2	0.2	0.2
	Starch+Soluble starch (1:1)	79.8	78.8	77.8	75.8	71.8
Amount consumed by <i>B. germanica</i> (mg)	in container A	-8	0	15	34	91
	B	8	-6	8	79	76
	C	6	-2	-2	74	79
	Total	6	-8	21	187	266

Table 6. Effect of glycerol in practical poison baits (II).

Weight composition of tablets (%)	Glycerol	0	1	2	4	8
	Boric acid	20	20	20	20	20
	Rice bran+Soluble starch (1:1)	80	79	78	76	72
Amount consumed by <i>B. germanica</i> (mg)	in container A	-1	6	7	59	88
	B	10	-3	11	85	97
	C	0	0	8	92	100
	Total	9	3	26	236	285

Table 7. Effect of glycerol in practical poison baits (III).

Weight composition of tablets (%)	Glycerol	0	5	10
	Boric acid	20	20	20
	Rice bran oil	16	16	16
	Soluble starch	10	10	10
	Starch	54	49	44
Amount consumed by <i>B. germanica</i> (mg)	in container A	0	24	69
	B	-2	4	62
	C	17	25	74
	Total	15	53	205

Table 8. Comparative effect of glycerol, maltose and sucrose in practical poison baits.

Adjuvant compound			Nothing	Sucrose	Maltose	Glycerol
Amount consumed by <i>B. germanica</i> (mg)	(Concentration of adjuvants in baits) (5%)	in container A	26	-7	5	152
		B	10	6	13	89
		Total	36	-1	18	241
	(10%)	in container C	-6	1	-2	115
		D	26	-3	-5	73
		Total	20	-2	-7	188
	(15%)	in container E	-13	9	15	43
		F	-7	2	42	71
		Total	-20	11	57	114

sects for 24 hours. The results are shown in Table 7.

The results in Tables 5, 6 and 7 clearly indicate that glycerol is highly effective in increasing the ingestion of the practical poison baits by *B. germanica* especially at higher concentrations.

Comparative effect of glycerol, maltose and sucrose.

The effect of the addition of glycerol to tablets was compared with that of maltose and sucrose. The formula of the baits was as follows: Glycerol, maltose, or sucrose X%, boric acid 20%, soluble starch 10%, starch 100-(30+X)%. In each test container, eight tablets consisting of two tablets of each composition of the same weight concentration were exposed to 30 female adults for 24 hours. Two test containers were used as repetitions for each concentration of the compounds. The results are shown in Table 8.

The insects most preferred glycerol-containing tablets at all concentrations tested.

DISCUSSION

Glycerol was strongly effective in the results of the filter paper method (Table 4), but the two glycols with three carbon atoms, related monoglycerides, and ethylene glycol were less or not effective (Table 1 and Fig. 1). The three hydroxyl groups in glycerol, therefore, seem to be essential for the strong feeding-stimulative activity of this compound.

Many polyol compounds with neighboring three or more carbon atoms each of which has a hydroxyl group, especially linear polyols, elicit the feeding response from the cockroaches. However, there are also many ineffective stereoisomers among them, especially among sugars (Table 1 and Tsuji, 1965). These findings may indicate that the configuration and naturally the shape of molecule are also important factors which determine the effectiveness of the compounds.

It is interesting to find that, a) among aldoses (pentoses and hexoses) L-arabinose and D-galactose with the same configuration at C₂-C₃-C₄ were strongly effective for all or

Fig. 1. Structure-activity relationships in glycerol and related compounds.
(Response data from Tables 1 and 2)

Fig. 2. Speculated effective arrangement in space of hydroxyl groups at three neighbouring carbon atoms in strongly feeding-stimulative compounds. (Response data from Table 1 and Tsuji, 1965.

B. g.: *Blattella germanica*, *P. a.*: *Periplaneta americana*,
P. f.: *Periplaneta fuliginosa*.)

one of the three species, whereas other stereoisomers tested were not so (Tsuji, 1965); b) glycerol can take all the imaginable arrangements in space of the three hydroxyl groups, and naturally can take the same arrangement of hydroxyl groups as at C₂-C₃-C₄ of L-arabinose; c) the same arrangement of hydroxyl groups as at C₂-C₃-C₄ of L-arabinose can occur more frequently in sugar alcohols than in corresponding aldoses, and many of the sugar alcohols tested were effective, whereas many of the aldoses tested were not effective (Table 1 and Tsuji, 1965); d) sorbitol which can take two sequential sets of the same arrangement of hydroxyl groups as at C₂-C₃-C₄ of L-arabinose, was exceptionally effective among the effective sugar alcohols (Tsuji, 1965).

One possible speculation is that glycerol, the effective sugar alcohols, and the effective aldoses stimulate the receptor of the cockroaches by combining with the same combining site which consists of sequential sets of structures corresponding in some way to the arrangement in space of hydroxyl groups at C₂-C₃-C₄ of L-arabinose (Fig. 2).

On the other hand, a few triglycerides are also feeding-stimulative (Table 3), though they have no free hydroxyl group in their structures. In these triglycerides, the cockroaches seem to respond to specific acyl groups which make a suitable size and shape

of glycerides. Several volatile fatty acids and their simple esters have been shown to act as strong attractants for cockroaches (Tsuji, 1966). Some of them are also feeding-stimulative. Accordingly, it is quite likely that acyl groups play an important role in both the volatile attractants such as fatty acids and their simple esters and the non- or less-volatile feeding stimulants such as triglycerides. The kind of acyl group may also be decisive importance in the effective monoglyceride, monoolein (Table 2), for monoglycerides have not three hydroxyl groups which are essential for the strong feeding-stimulative activity of glycerol (Fig. 1).

The results obtained by the bait method with the German cockroach show that glycerol is quite effective as a feeding-stimulant in practical poison baits (Tables 5, 6 and 7), and that glycerol is much more effective than maltose of the same weight concentrations in baits (Table 8).

SUMMARY

Some simple polyols and related compounds were assayed by a filter paper method for the feeding-stimulative effect on the three species of cockroaches, *Periplaneta americana*, *P. fuliginosa* and *Blattella germanica*. Glycerol was further tested by a bait method

for *B. germanica*.

I. The results obtained by the filter method are as follows.

1) Glycerol is highly effective, but ethylene glycol, 1,2-propanediol and 1,3-propanediol are not.

2) The three hydroxyl groups in glycerol, therefore, seem to be essential for the strong activity of this compound.

3) Many compounds with neighboring three or more carbon atoms each of which has a hydroxyl group, especially sugar alcohols, are effective. However, there are also many ineffective stereoisomers among them, especially among sugars (Tsuji, 1965).

4) Therefore, not only the number of hydroxyl groups but the configuration of molecule is also one of the important factors which determine the effectiveness of the compound.

5) It is interesting to know that, a) among aldoses (pentoses and hexoses) L-arabinose and D-galactose with the same configuration at C₂-C₃-C₄ were strongly effective for all or one of the three species, whereas other stereoisomers tested were not (Tsuji, 1965); b) glycerol can take all the imaginable arrangements in space of the three hydroxyl groups, and naturally can take the same arrangement of hydroxyl groups as at C₂-C₃-C₄ of L-arabinose; c) the same arrangement of hydroxyl groups as at C₂-C₃-C₄ of L-arabinose can occur more frequently in sugar alcohols than in corresponding aldoses, and many of the sugar alcohols tested were effective, whereas many of the aldoses tested were not effective; d) sorbitol which can take two sequential sets of the same arrangement of hydroxyl groups as at C₂-C₄-C₄ of L-arabinose was exceptionally effective among the effective sugar alcohols.

6) One possible speculation is that glycerol, the effective sugar alcohols, and the effective aldoses stimulate the receptor of the cockroaches by combining with the same combining site which consists of sequential sets of structures corresponding in some way to the arrangement in space of hydroxyl groups at C₂-C₃-C₄ of L-arabinose.

7) A few glycerides, including monoolein, triolein and tricaprins, are somewhat effective, though they have not three hydroxyl groups.

In these glycerides, the cockroaches seem to respond to specific acyl groups in their structures.

II. The results obtained by the poison bait method with the German cockroach show that glycerol is quite effective as a feeding-stimulant in practical poison baits, and is much more effective than maltose, the strongest feeding-stimulative sugar.

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摘 要

ゴキブリ類に対するグリセロール及び 関連物質の摂食行動誘起作用

糖の一部や糖アルコールの一部がゴキブリの摂食行動を起させることはすでに報告した (辻 1965, 辻・大野 1969)。今回はより単純な多価アルコール其他の作用を検討し、これらの化合物が有効であるための条件について若干考察した。

I. 前報及び今回のワモンゴキブリ, クロゴキブリ, チャバネゴキブリを用いた濾紙法による結果から次のことがいえよう。1) グリセロールは極めて有効である。2) この強力な作用のためにはグリセロールのもつ3個の水酸基の全部が必要である。グリコール類は無効であり, 単純な飽和脂肪酸の α -モノグリセリドもモノアセチンがごくわずかの作用を示す以外は全く無効であった。3) グリセロールのように隣接した3個又はそれ以上の炭素に夫々1個の水酸基をもつ化合物 (特に糖アルコール) には有効なものが多いが, それらの立体異性体 (特に糖で) で無効なものも多い (但しチャバネゴキブ

リに対しては他種に対してより無効のことが多い). 4) 従って水酸基の数ばかりでなく, その立体的な配置及び化合物全体の形態も又重要な条件と思われる. 5) 一方, テストしたアルドース (5 及び 6 炭糖) のうちで, $C_2C_3C_4$ に於ける立体配置の等しい L-アラビノース, D-ガラクトースだけが 3 種又は 1 種 (クロゴキブリ) のゴキブリに極めて有効で他の異性体が無効か少効であること: L-アラビノースの $C_2C_3C_4$ に於けると同様の立体配置はアルドースよりもそれらに対応する糖アルコールで末端のメチロールの回転によつて成立しやすく多くの糖アルコールは有効であり, 他方多くのアルドースは無効であること: L-アラビノースの $C_2C_3C_4$ と同様の配置がソルビトールの $C_6C_5C_4$ 及び $C_3C_2C_1$ に連続かつそれぞれ独立に成立し, この化合物がゴキブリ 3 種

に対して糖アルコール中で, ずばぬけた効果を示すこと: 又上記と同様の配置をグリセロールが容易にとり得ること: の諸事実は極めて暗示的である. 6) あるいは, ゴキブリ 3 種に於いてグリセロールを感受するレセプターはその分子レベルでこれらの有効化合物に対して共通であり, L-アラビノースの $C_2C_3C_4$ の立体配置に対応した構造の連続によつて構成されているかも知れない. 7) よく知られた油脂成分であるオレイン酸其他のグリセライドも若干有効であるが, この場合ゴキブリはむしろ特定のアシル基に反応していると考えられる.

II. チャバネゴキブリを用いた実用的なベイトによる試験結果によれば, グリセロールは今まで知られた最も強い効果をもつ 2 糖類のマルトース以上に強力な効果を示した.

Recombination values between the white eye and factors for resistance to Dieldrin, Abate, Fenthion and Fenitrothion in larvae of *Culex pipiens pallens* Coquillett

By

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Linkage relationships of factors for resistance to fenitrothion (Sumithion), fenthion, malathion and Abate have been studied in the strains of *Culex pipiens pallens* and these factors were relatively closely linked with each other in linkage group 2 (Tadano, 1960 a & b, Tadano, 1970). The purpose of this study is to calculate genetical recombination values between *w* (white eye) and factors for resistance to dieldrin, fenthion, fenitrothion and Abate in the larvae of the *C. p. pallens* strains of the same origin as the strains used in the above studies.

MATERIALS AND METHODS

The insecticides used in this study were as follows: dieldrin, Abate (*O, O, O', O'*-Tetramethyl *O, O'*-thiodi-*p*-phenylene phosphorothioate), fenthion [Baytex, *O, O*-Dimethyl-*O*-4-(methyl mercapto)-3-methyl phenyl thionophosphate] and fenitrothion [Sumithion, *O,*

O-Dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate].

The following three organophosphate-resistant strains of *C. p. pallens*, which originated from the NA colony (Suzuki, 1968), the *w* and *ru* (ruby eye) colonies were employed in this experiment.

NADR : derived from the NA colony by larval selection with dieldrin for 5 generations after 10 generations of selection with Abate. The LC_{50} values were 0.095 ppm for Abate and 16 ppm for dieldrin.

NAFR : pressurized with fenthion at its larval stage for 7 generations after 10 generations of larval selection with Abate. The LC_{50} were 0.068 ppm for Abate and 0.15 ppm for fenthion.

NFSR : selected with fenthion for 7 generations following the larval pressurization with fenitrothion for 6 generations. The LC_{50} values were 0.16 ppm for the fenthion and 0.3 ppm for fenitrothion.

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