Attractants for *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae): Dimethyl Succinate, Glutarate, and Adipate

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Dimethyl esters of succinic, glutaric, and adipic acids have been isolated, probably as artifacts, from the steam volatiles of rolled oats and have been identified by NMR and GC–MS. The isolated compounds, and also their authentic counterparts, strongly attract *Oryzaephilus surinamensis* (L.) (sawtoothed grain beetle) adults in a laboratory pitfall chamber bioassay. Other bioassays utilizing commercial cardboard monitoring traps also demonstrated that the three esters were attractive. The attractancy of an oat oil–wheat germ oil–mineral oil mixture was enhanced by incorporating small amounts of authentic dimethyl glutarate into the mixture.

Previous work in our laboratory (Freedman et al., 1982; Mikolajczak et al., 1983, 1984) and by other workers (Pierce et al., 1981) demonstrated that oats generate a number of chemical stimuli that induce responses in *Oryzaephilus surinamensis*. Some of the materials responsible such as triglycerides and free fatty acids stimulate aggregation, whereas two others, identified as (*E*)-2-nonenal and (*E*,*E*)-2,4-nonadienal, strongly attract the beetles over a distance (Dethier et al., 1960) in pitfall bioassay chambers. These two aldehydes (and a number of others) were isolated from fractions of rolled oat steam volatiles that eluted from a silica gel column with ether–pentane (2:98 and 5:95) (fractions 12–16 in Figure 1, Mikolajczak et al., 1984). Slightly more polar fractions (numbered 22–25) were eluted from the same column with ether–pentane (1:3) and were shown by gas chromatography (GC) to contain varying proportions of a number of common constituents. Although only fraction 25 produced a significant response in the Petri dish bioassay, all four fractions were attractive to the sawtoothed grain beetle in the pitfall bioassay. Our present study was undertaken to isolate from combined fractions 22–25 the chemical entities responsible for the observed beetle responses and to determine their structures.

MATERIALS AND METHODS

Insect Rearing. Sawtoothed grain beetles were reared as previously described (Mikolajczak et al., 1984).

Pitfall Chamber Bioassays. The two types of pitfall chambers developed by Phillips and Burkholder (1981) and also by Pierce et al. (1981) were both used at various times; data in Tables II and III were obtained with the Pierce chamber. Pentane solutions (10 μL) of appropriate concentrations of test stimuli were applied to test paper disks, and 10 μL of solvent only was applied to control disks. The bioassays utilized 5–7-day starved beetles and were conducted in darkness at 27 ± 1 °C and 60 ± 5% RH for 1 h. Beetle responses to treated disks vs. control disks were converted to "percent response" (Tamaki et al., 1971; Nara et al., 1981), which is defined as 100 $(T - C)/N$; T and C are the number of insects in the vials containing the treated and control disks, respectively, and N is the total number of insects used. Insect response data were treated statistically as reported earlier (Mikolajczak et al., 1984).

Cardboard Trap Bioassay 1. This bioassay utilized traps with a small plastic stimulus cup surrounded by a folded, corrugated cardboard holder (Barak and Burkholder, 1985). It is marketed as Storgard by Zoëcon Corp. The prototype plastic cup was somewhat soluble in the pure dimethyl esters so a 27 × 12 mm plastic vial lid was placed inside the furnished cup to contain the sample. In

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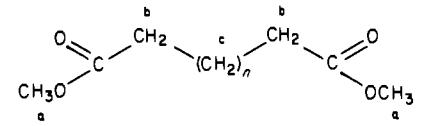
Table I. NMR[a] Analysis of Dicarboxylic Acid Methyl Esters Isolated from Oats



peak	identity	n	δ^b (no. of protons) a	b	c
A	dimethyl succinate	0	3.69 s (6 H)	2.63 s (4 H)	
B	dimethyl glutarate	1	3.66 s (6 H)	2.37 t (4 H)	1.94 q (2 H)
C	dimethyl adipate	2	3.66 s (6 H)	2.32 t (4 H)	1.65 m (4 H)

[a] 90 MHz in $CDCl_3$. [b] Key: s = singlet, t = triplet, q = quartet, m = multiplet.

Table II. Percent Response[a] of *Oryzaephilus surinamensis* to Stimuli Isolated from Rolled Oat Volatiles

stimulus	GC retentn index[b]	amt in combined 22–25 (GC area %)	% response[c,d] isolated	diluted 1:10	diluted 1:100
combined col chromatogr fractions 22–25			3	49**	
prep GC fractions					
peak A (dimethyl succinate)	1010	36	27**	53**	44**
peak B (dimethyl glutarate)	1111	40	−20*	51**	44**
peak C (dimethyl adipate)	1213	7	19	37**	−7

[a] Pitfall chamber bioassay. [b] Fused silica DB-1 column, 15 m × 0.32 mm i.d. [c] (*) and (**) indicate significance at $p < 0.05$ and $p < 0.01$, respectively, based on χ^2 analysis. [d] Based on four replicates of 25 insects each.

Table III. Percent Response[a] of *Oryzaephilus surinamensis* to Authentic Dimethyl Esters

stimulus	% response[b,c] 100 μg	10 μg	1 μg	0.1 μg	0.01 μg
dimethyl succinate	−10	17*	30**	25**	−7
dimethyl glutarate	−18*	36**	33**	24**	−3
dimethyl adipate	3	31**	32**	9	

[a] Pitfall chamber bioassay. [b] (*) and (**) indicate significance at $p < 0.05$ and $p < 0.01$, respectively, based on χ^2 analysis. [c] Based on eight replicates of 25 insects each.

bioassays comparing (a) oat oil (Quaker Oats Co., Barrington, IL), (b) wheat germ oil (Viobin Corp., Monticello, IL), (c) mineral oil, or (d) a mixture of equal amounts of these three oils against dimethyl glutarate, 0.5 mL of an oil sample was placed in the test trap cup and a like amount of dimethyl glutarate in the control trap cup. A test and a control trap were then positioned in diagonally opposite corners of a 51 × 51 × 6.5 cm stainless-steel tray (2.5 cm from the Teflon-coated sides). One hundred 3–7-day starved *O. surinamensis* beetles were introduced into the center of the tray. The four replicate trays were stored for 3 days in a 5 × 8 × 3 m windowless room with good air circulation and having one open doorway on a lighted hallway; temperatures ranged from 22 to 30 °C and RH from 40 to 80%. The trays were placed in the darkest area of the room and were positioned so that no two traps were in the same position relative to the doorway. Insects were then counted, with those actually found in the stimulus cups being counted separately from those found within the corridors of the corrugated cardboard portion of the traps. Traps were not reused.

Cardboard Trap Bioassay 2. Two experiments were conducted in the same type of apparatus as bioassay 1 except that cups made from a different plastic were used. One hundred *O. surinamensis* adults that had been starved 4–5 days were used in each of four replicates. The trays were covered loosely and held in darkness at 27 ± 1 °C and 60 ± 5% RH for 16 h. The first experiment was conducted with test stimuli containing 1.0, 1.5, and 2.0 μL of dimethyl glutarate, respectively, in 0.5 mL of the three-oil mixture; the second experiment was the same as the first except that test stimuli containing 1.0, 3.0, and 10.0 μL of dimethyl glutarate, respectively, were used. In each case, the control trap cup held 0.5 mL of the mixed oils. Insect response means were compared by Duncan's new multiple-range test.

Isolation of Volatiles—Large Scale. Rolled oat volatiles were isolated as detailed previously (Mikolajczak et al., 1984). One batch of oats (Quaker Old-Fashioned) was steeped in pentane and one in dichloromethane. After removal of most of the solvent, these crude extracts were subjected to a simultaneous steam distillation–extraction using a Likens–Nickerson head (Likens and Nickerson, 1964) with dichloromethane as the extracting solvent. Most of the solvent was distilled from the volatiles, and the residual dichloromethane was removed by azeotropic distillation with methanol; the methanol was then azeotropically distilled with pentane. The two resulting pentane solutions of volatiles were each further resolved into 30 fractions by silica gel column chromatography. Bioassay data and GC analysis of all fractions indicated that it was feasible to combine corresponding fractions from the pentane and dichloromethane extracts, thus providing 30 fractions.

Isolation of Volatiles—Artifact Detection. Two 800-g samples of rolled oats were extracted, one with pentane and one with dichloromethane, and the extracts were steam distilled as above. Half of each dichloromethane solution from the steam distillation–extractions was distilled down using methanol as indicated above; the other half was processed without adding methanol. Each of the four oat volatiles samples thus obtained was separated by column chromatography into 30 fractions, and appropriate fractions were analyzed for the presence of the dicarboxylic acid methyl esters by GC–MS.

Gas Chromatography. Analytical and preparative GC equipment, conditions, and procedures, including retention index calculations and fraction collection and recovery, were as outlined previously (Mikolajczak et al., 1984) with the following modifications. Analytical chromatograms and retention indices were obtained by operating the fused silica DB-1 column at 60 °C for 3 min and then programming to 270 °C at 5 °C/min and holding. For the preparative GC mode, a 175 × 0.4 cm i.d. glass column packed with 3% Dexsil 300 on 100–120 mesh Chromosorb W-AW (Alltech Associates) was used. Operating parameters were as follows: column temperature, 60 °C for 3 min and then programmed to 350 °C at 8 °C/min; injector, 300 °C; detector, 350 °C; helium flow, 25 mL/min at 20 psi inlet pressure; effluent split ratio, 10:1.

Gas Chromatography–Mass Spectrometry (GC–MS). A 15 m × 0.32 mm i.d. fused silica DB-1 (1-μm film thickness) column coupled to a Kratos MS 30 mass spectrometer was employed for GC–MS analyses of the isolated dimethyl esters. The column temperature was held at 60 °C for 3 min and was then programmed at 7 °C/min to 220 °C. Mass spectra were obtained at 70 eV. A 40 m × 0.32 mm i.d. fused silica DB-1 column, operated at the same conditions and coupled to a Finnigan MAT 4535/TSQ instrument, was used in the artifact detection experiments.

Nuclear Magnetic Resonance (NMR). ^{1}H NMR spectra were obtained on $CDCl_3$ solutions at 90 MHz with a Bruker WH-90 spectrometer. Chemical shifts were measured relative to the $CHCl_3$ signal set at δ 7.25.

Authentic Chemicals. Authentic samples of dimethyl succinate (dimethyl butanedioate), dimethyl glutarate (dimethyl pentanedioate), and dimethyl adipate (dimethyl hexanedioate) were purchased from Aldrich Chemical Co.

RESULTS AND DISCUSSION

Structure Determination. The four fractions (22–25) from column chromatography of oat volatiles were combined to provide sufficient material for preparative GC procedures. Capillary GC analysis of the resulting mixture presented a complex picture, but the three components that were isolated and labeled peaks A–C accounted for 83% of the total sample. The purities of the isolated components determined by capillary GC were as follows: A, 97% B, 99%; C, 99%.

The ^{1}H NMR spectra of the unknowns (Table I) each contained a singlet appropriate for methyl ester methoxy protons: δ 3.69 for A and δ 3.66 for B and C. A singlet at δ 2.63 was the only other signal in the spectrum of A. It contained two-thirds as many protons as the δ 3.69 singlet, thereby strongly suggesting that A was dimethyl succinate. In addition to the methoxy signal, peak B produced a triplet at δ 2.37 and a five-line pattern centered at δ 1.94, both of which showed evidence of fine splitting. If the area of the δ 1.94 signal represents two protons, the δ 2.37 signal is equivalent to four protons (on carbons adjacent to carboxyl groups), and the methoxy signal contains six protons. This spectrum is identical with that of authentic dimethyl glutarate. The four protons on carbons adjacent to carboxyl groups in peak C appear as a broadened triplet at δ 2.32. The conclusion that C is dimethyl adipate is drawn from the fact that its methylene protons β to carboxyl groups give a four-proton multiplet at δ 1.65.

Mass spectral data for peaks A–C are consistent with the proposed structures although molecular ions were not observed. Peak A gave m/z 115 (M − OCH_3, 100%) and m/z 87 (M − $OCOCH_3$, 16%) as major ions. The loss of $-OCH_3$ was also a preferred fragmentation for B to give m/z 129 (60%) and for C to give m/z 143 (75%). Cleavage of the $-OCOCH_3$ grouping (M − 59) from B and C yielding m/z 101 (40%) and m/z 115 (22%), respectively, was

prevalent, but both compounds also showed a loss of the neutral fragment, acetic acid (M − 60), thus producing intense ions of m/z 100 (65%) from B and of m/z 114 (100%) from C. A similarity index, generated by computer comparison of these data with a mass spectra library, strongly suggested that these components had the indicated structures. Authentic dimethyl succinate, glutarate, and adipate gave GC retention indices in agreement with those listed in Table II for peaks A–C.

In the original isolation procedure, after steam distillation of the volatiles, it was necessary to have them dissolved in pentane instead of dichloromethane for column chromatography, but complete evaporation of solvent might haved caused some loss of the more volatile constituents. Since methanol forms a low-boiling azeotrope with dichloromethane and also with pentane, it was used to remove residual dichloromethane and then was itself removed by distillation with pentane, leaving the volatiles in pentane. It was later discovered that dichloromethane also codistills with pentane.

Although Heydanek and McGorrin (1981a,b) have reported the presence of diethyl fumarate in oats, dimethyl esters are not known to occur. For this reason, and also because methanol was involved in the isolation procedure, possible artifact formation was investigated. Small-scale isolations of pentane-extracted and dichloromethane-extracted volatiles were done, and the samples were worked up both with methanol and without methanol. None of the three dimethyl esters could be detected by GC–MS analysis of fractions obtained from the methanol-free workup procedure, but detectable amounts of dimethyl succinate and dimethyl glutarate were found by GC–MS in corresponding fractions that had been treated with methanol. Treatment of the fractions containing none of the three diesters with acidic methanol, followed by GC–MS, increased the content of succinate and glutarate considerably over that of the samples treated only with methanol; dimethyl adipate was also present. This experiment was carried out a second time with the same results. It is not known what form these acids occur as in the extracts, but the dimethyl esters isolated were probably artifacts.

Petri Dish and Pitfall Trap Bioassays. Of the four column chromatographic fractions that were combined on the basis of their GC profiles for this investigation, only fraction 25 had demonstrated activity in the Petri dish bioassay (Mikolajczak et al., 1984). The resulting combined sample diluted 1:10 elicited significant attraction of the sawtoothed grain beetle in the pitfall chamber (Table II). Peaks A–C as isolated by preparative GC also required dilution to achieve optimal attraction in this bioassay. In order to prevent possible loss of sample, the solvent was never completely removed; hence, the actual quantity tested was not determined precisely. No dilutions beyond 1:100 were made.

After the identities of A–C were learned, their commercially available authentic counterparts were assayed using *O. surinamensis* in the pitfall chamber, and all were attractive. Doses from 0.01 to 100 μg per disk were tested; the results appear in Table III. The approximate magnitude of the responses and the effective dose range shown here are quite similar to those reported for (*E*)-2-nonenal and (*E*,*E*)-2,4-nonadienal in pitfall bioassays (Mikolajczak et al., 1984).

Cardboard Trap Bioassays. Storgard cardboard monitoring traps were employed to present authentic dimethyl glutarate to *O. surinamensis* under more "fieldlike" conditions; dimethyl glutarate was chosen because it was as attractive as any of the three esters and also because it had intermediate volatility.

Table IV. Percent Response[a] of *Oryzaephilus surinamensis* to Various Oils vs. Dimethyl Glutarate as the Control

stimulus	% resp[b,c] of insects	
	stimulus cup	entire trap
oat oil	62**	64**
wheat germ oil	60**	69**
mineral oil	−29**	−29**
1:1:1 mixture of above oils	54**	66**

[a] Cardboard trap bioassay 1; 0.5-mL neat samples. [b] (**) indicates significance at $p < 0.01$, based on χ^2 analysis. [c] Based on four replicates of 100 insects each.

Table V. Response[a] of *Oryzaephilus surinamensis* to Mixed Oils Containing Various Concentrations of Added Dimethyl Glutarate

stimulus	insects[b]	
	stimulus cup	entire trap
	Experiment 1	
control[c]	7.25a	13.0a
1.0 μL[d]	12.25ab	18.25ab
1.5 μL[d]	18.75b	26.25bc
2.0 μL[d]	22.0b	29.5c
	Experiment 2	
control[c]	10.75ab	14.5a
1.0 μL[d]	14.25b	22.5b
3.0 μL[d]	12.75b	22.5b
10.0 μL[d]	8.0a	21.25b

[a] Mean number of insects responding; cardboard trap bioassay 2. [b] Means followed by the same letter do not differ significantly ($p < 0.05$) according to Duncan's new multiple-range test. [c] 0.5 mL of an oil mixture containing equal amounts of wheat germ oil, oat oil, and mineral oil. [d] In 0.5 mL of oil mixture.

Since wheat germ oil and oat oil are known attractants for certain stored-product insects (Tamaki et al., 1971; Nara et al., 1981; Freedman et al., 1982), the response of *O. surinamensis* was determined when the insects were given a choice between neat dimethyl glutarate and these oils in the cardboard traps. The data in Table IV demonstrate that although the beetles were somewhat attracted to dimethyl glutarate vs. mineral oil, they much preferred the neat crude oils, or a mixture of them in mineral oil, as opposed to dimethyl glutarate. Bioassays of dimethyl glutarate vs. mineral oil and the oil mixture using 3-day starved beetles instead of 5- to 7-day starved beetles yielded results comparable to those in Table IV.

The results presented in Table V, experiment 1, show conclusively that 1.5–2.0 μL of dimethyl glutarate added to 0.5 mL of a 1:1:1 wheat germ oil–oat oil–mineral oil mixture significantly increased the insect catch in both the stimulus cup and the cardboard portion of the traps. In this experiment and the next, the four traps (three concentrations of stimulus plus control) were all placed in one tray. Since the observed attractancy increased with concentration up to 2 μL, experiment 2 was conducted to show the effect of higher concentrations. In this case, there were no significant differences in total trap catches among the concentrations tested, but all were significantly higher than the control. However, the number of insects entering the cups decreased as the concentration of dimethyl glutarate increased. This decrease may indicate a trend toward repellency (also seen in Table III) of dimethyl glutarate at higher concentrations although a repellent effect was not observed when mineral oil was tested with a neat dimethyl glutarate control (Table IV). The reasons for this apparent anomaly are not evident, but a contributing

factor could be that three different assays with three different controls are involved.

Summary. We have shown that dimethyl succinate, dimethyl glutarate, and dimethyl adipate strongly attract the sawtoothed grain beetle in laboratory bioassays. However, these esters as isolated here from rolled oats are probably artifacts, whose formation was precipitated by the use of methanol in the isolation procedure.

Dimethyl glutarate enhances the attractancy of a wheat germ oil–oat oil–mineral oil mixture considerably when added to the mixture in 1.5–10.0 μL amounts. These chemically stable constituents may find applications as attractants, either alone or in conjunction with food-type stimuli now used with the Storgard cardboard monitoring trap.

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Registry No. Dimethyl glutarate, 1119-40-0; dimethyl succinate, 106-65-0; dimethyl adipate, 627-93-0.

LITERATURE CITED

Barak, A. V.; Burkholder, W. E. *Agric., Ecosyst. Environ.* **1985**, *12*, 207.

Dethier, V. G.; Brown, L. B.; Smith, C. N. *J. Econ. Entomol.* **1960**, *53*, 134.

Freedman, B.; Mikolajczak, K. L.; Smith, C. R., Jr.; Kwolek, W. F.; Burkholder, W. E. *J. Stored Prod. Res.* **1982**, *18*, 75.

Heydanek, M. G.; McGorrin, R. J. *J. Agric. Food Chem.* **1981a**, *29*, 950.

Heydanek, M. G.; McGorrin, R. J. *J. Agric. Food Chem.* **1981b**, *29*, 1093.

Likens, S. T.; Nickerson, G. B. *Proc. Am. Soc. Brew. Chem.* **1964**, 5–13.

Mikolajczak, K. L.; Freedman, B.; Zilkowski, B. W.; Smith, C. R., Jr.; Burkholder, W. E. *J. Agric. Food Chem.* **1983**, *31*, 30.

Mikolajczak, K. L.; Zilkowski, B. W.; Smith, C. R., Jr.; Burkholder, W. E. *J. Chem. Ecol.* **1984**, *10*, 301.

Nara, J. M.; Lindsay, R. C.; Burkholder, W. E. *J. Agric. Food Chem.* **1981**, *29*, 68.

Phillips, J. K.; Burkholder, W. E. *J. Econ. Entomol.* **1981**, *74*, 539.

Pierce, A. M.; Borden, J. H.; Oehlschlager, A. C. *Can. J. Zool.* **1981**, *59*, 1980.

Tamaki, Y.; Loschiavo, S. R.; McGinnis, A. J. *J. Agric. Food Chem.* **1971**, *19*, 285.

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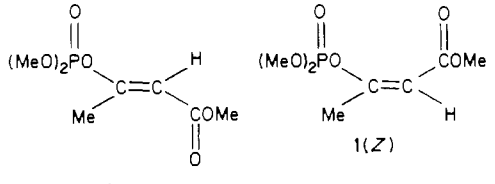
β-Cyclodextrin Inclusion Complex of Mevinphos

Geoff Boocock and Patrick Camilleri*

Mevinphos [2-(methoxycarbonyl)-1-methylvinyl dimethyl phosphate] is a contact and systemic insecticide. The technical product contains at least 60% w/w and up to 20% w/w of the *E* and *Z* isomers, respectively. The *E* isomer is the more active ingredient. In this study, we report the selective inclusion of the *E* isomer in β-cyclodextrin to give a solid formulation that is insecticidally active and dissolves rapidly in water to give a clear solution.

Mevinphos was introduced by Shell Development Co. in 1953 under the trade name Phosdrin and is now a well established organophosphorus insecticide with a wide range of activity. It is fast acting and highly effective at low dosage rates (Shell International Chemical Co., 1976). Phosdrin is water miscible and systemic in plants and also has fumigant, acaricidal, and ovicidal properties. The active component in Phosdrin is 2-(methoxycarbonyl)-1-methylvinyl dimethyl phosphate (1) which can exist as the *E* isomer, mp 21 °C, or as the *Z* isomer, mp 7 °C. Phosdrin contains at least 60% w/w and up to 20% w/w of the *E* and *Z* isomers, respectively.



Phosdrin has a high mammalian toxicity both orally (rat LD_{50} = 2.9–12 mg/kg) and percutaneously (rat LD_{50} = 1.9 mg/kg) (Shell International Chemical Co., 1976). The percutaneous toxicity is also high when the toxicant is formulated as an emulsifiable concentrate. Transformation of liquid Phosdrin into a solid form that could be formulated as pellets or granules should make it much safer to handle during the preparation of the spray liquid. During our search for such a formulation we discovered that treatment of Phosdrin with a solution of β-cyclodextrin in water gave rise to an inclusion complex that contained exclusively the *E* isomer. This form of encapsulated 1 showed excellent insecticidal activity. In this text we shall refer to the encapsulated form of Phosdrin in β-cyclodextrin by the abbreviation PCDC (Phosdrin cyclodextrin cryptate).

Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, U.K.

EXPERIMENTAL SECTION

Synthesis of Phosdrin β-Cyclodextrin Cryptate. β-Cyclodextrin (8.2 g) was dissolved in distilled water (100 mL) at 59 °C. The warm solution was slowly treated dropwise with stirring with technical Phosdrin (17.2 g). The mixture was cooled in an ice bath with continued stirring for 3 h. The white precipitate formed was filtered off, washed with ice water (3 × 7 mL), and dried in a desiccator to give a yield of 9.1 g of PCDC.

Analyses. *Chromatography.* Phosdrin was analyzed by gas–liquid chromatography with a flame ionization detector. The separation of the *E* and *Z* isomers was

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