Production of (5*R*,6*S*)-6-Acetoxy-5-hexadecanolide, the Mosquito Oviposition Pheromone, from the Seed Oil of the Summer Cypress Plant, *Kochia scoparia* (Chenopodiaceae)

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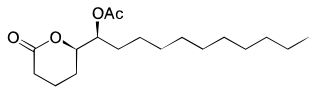
The oviposition pheromone for the pathogen-vectoring mosquitoes in the genus *Culex* (Diptera: Culicidae), that is, (5*R*,6*S*)-6-acetoxy-5-hexadecanolide, is efficiently synthesized, in admixture with the inactive (5*S*,6*R*) enantiomer (∼33% w/w), from the fixed oil extracted from the seeds of the summer cypress plant, *Kochia scoparia* (Chenopodiaceae), cultivated on an industrial scale. Oviposition bioassays using gravid females of *Culex quinquefasciatus*, a vector of filariasis in human beings, showed that the product was attractive, with activity comparable to that of a pure synthetic sample containing the same amount of the active enantiomer. Production of the pheromone in the form of a biologically active crude material via a cheap and renewable plant suitable for development as a new industrial crop provides the basis for control of *Cx. quinquefasciatus* and other congeneric vectors of pathogens in resource-poor areas of the world.

Keywords: *Kochia scoparia; seed oil; Culex quinquefasciatus; (5R,6S)-6-acetoxy-5-hexadecanolide; mosquito; oviposition; pheromone*

INTRODUCTION

Of the many blood-sucking and biting insects belonging to the order Diptera, mosquitoes (Culicidae) pose the greatest threat to public health because of their ability to act as vectors of causative agents for diseases such as malaria, dengue, yellow fever, encephalitis, and filariasis, which afflict many millions of people worldwide, particularly in sub-Saharan Africa (American Association for the Advancement of Science, 1991; Giles and Warrell, 1993; WHO/CTD, 1998). In terms of numbers of affected individuals and level of mortality, malaria and encephalitis are of greatest significance, together affecting nearly one-third of the world's population (1.5 billion people) in some 90 countries, mostly within Africa. For filariasis, it is estimated that, of the 450 million people that are susceptible, >15 million people are currently infected, with 950000 new cases occurring annually (Reeves and Milby, 1990). Efficient techniques for vector surveillance and control are therefore of paramount importance.

The mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) is responsible for transmission of *Wuchereria bancrofti*, the causative agent of human filariasis and the St. Louis encephalitis virus and other arboviruses in the United States (Reisen et al., 1992). Gravid *Cx. quinquefasciatus* females use olfactory cues for the location of suitable egg-laying sites. The main cue is a volatile pheromone, released by mature egg rafts (Osgood, 1971; Osgood and Kempsler, 1971; Starratt and Osgood, 1973), which acts in combination with water-based pollutants such as 3-methylindole produced, for example, via bacterial fermentation [Millar et al., 1992; Mordue (Luntz) et al., 1992]. The first step in developing new strategies for control of *Cx. quinquefasciatus* using such semiochemicals (behavior- or development-modifying chemicals) was the identification of the oviposition pheromone as (5*R*,6*S*)-6-acetoxy-5-hexadecanolide (**1a**)



1a: mosquito oviposition pheromone

(Laurence and Pickett, 1982, 1985; Laurence et al., 1985). Laboratory and field trials in nine countries and three continents, using synthetic material (Dawson et al., 1990) containing an equal mixture of all four possible stereoisomers, that is, (5*R*,6*R*), (5*R*,6*S*), (6*R*,5*R*), (6*R*,6*S*), have demonstrated the efficacy of the pheromone in attracting *Culex* spp. mosquitoes (Otieno et al., 1988). Despite the presence of three inactive unnatural isomers, the biological activity of the naturally occurring isomer is unaffected.

The characterization of the oviposition pheromone **1a** has provided the impetus for several asymmetric syntheses and large scale racemic routes, respectively, as a challenge for synthetic chemists and to provide material for field testing (Laurence and Pickett, 1982;

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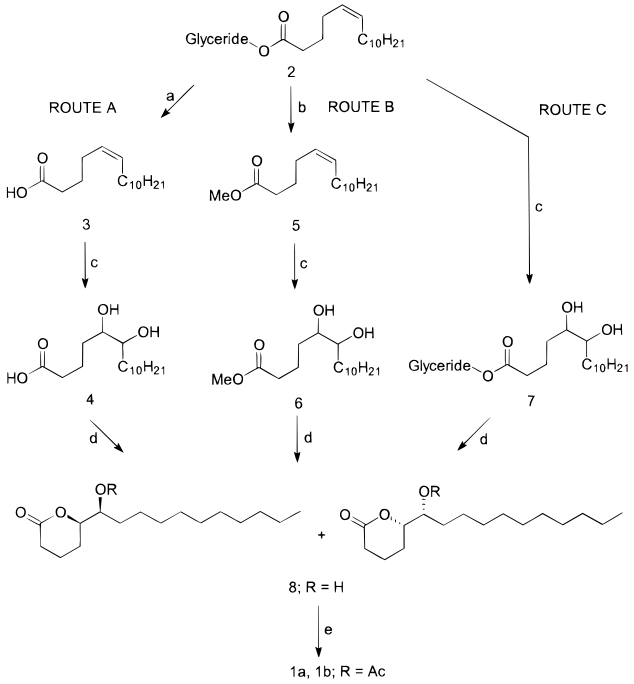
Scheme 1[a]



[a] Reagents: (a) 2 M NaOH, C_6H_{12}, reflux, then 6 M HCl; (b) BF_3·MeOH, reflux; (c) *t*-BuOOH, OsO_4 (cat.), 0−25 °C; (d) *p*-TSA, C_6H_6, reflux; (e) Ac_2O, C_5H_5N, 25 °C.

Fuganti et al., 1982; Mori and Otsuka, 1983; Sato et al., 1984; Lin et al., 1985; Machiya et al., 1985; Ochiai et al., 1985; Barua and Schmidt, 1986; Jefford et al., 1986; Ko and Eliel, 1986; Dawson et al., 1990; Coutrout and Bomont, 1994; Gravierpelletier et al., 1995; Henkel et al., 1997). Although the various synthesis routes cited above can provide multigram quantities of **1a**, the economic circumstances of the less developed countries make control strategies for *Cx. quinquefasciatus* based on the synthetic pheromone unaffordable. Here we describe a rapid, semi-biotechnological approach to pheromone production, as a component of a crude but chemically defined product, from the fixed oil extracted from seeds of the summer cypress, *Kochia scoparia* (Chenopodiaceae). Generally, seeds from the family Chenopodiaceae are not rich in oil (Kleinman and Earle, 1972). However, the fatty acid composition of the oil from some members of this family such as spinach (*Spinocea oleoracea*), Russian thistle (*Salsola pestifer*), *Bassia hypsofolia*, *Kochia prostata*, and *Sieda setigera*, as well as *K. scoparia*, is reported to contain 0.2−5.0% 5-hexadecenoic acid (Kleiman and Earle, 1972). Although *K. scoparia* seed oil does not possess the highest amount (w/w) of the acid, the plant was chosen for pheromone production because of its ease of cultivation and prolific seed production [11000 kg of seed/hectare (ha)]. It represents a good alternative as a grazing or forage crop and can be baled and ensiled. *K. scoparia* grows rapidly (1−2.5 cm/day) and vigorously; it tolerates very dry conditions and is found in most countries afflicted with *Culex* spp. mosquitoes (Garduno, 1993). Three different approaches are utilized for the preparation of a common intermediate, (5*R*,6*S*)-6-hydroxy-5-hexadecanolide (**8**), from glycerides of (*Z*)-5-hexadecenoic acid (**2**), with a final acetylation step to give the desired enantiomerically enriched 6-acetoxyhexadecanolide **(1a)** (see Scheme 1).

MATERIALS AND METHODS

General. ^{1}H NMR spectra were recorded in deuteriochloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal standard, at 400 MHz on a JEOL GNX spectrometer. ^{13}C NMR spectra were obtained from the JEOL spectrometer at 100 MHz also using TMS as the internal standard. Chemical shifts (δ_H and δ_C) are reported in parts per million (ppm) from the internal standard (TMS = 0.00). Gas chromatography (GC) was carried out using a Hewlett-Packard 5890 gas chromatograph equipped with a cold on-column injector, a flame ionization detector (FID), and a 50 m × 0.32 mm i.d. HP-1 capillary column. The oven temperature was maintained at 40 °C for 2 min and programmed at 10 °C/min to 250 °C. The carrier gas was hydrogen. Gas chromatography/mass spectrometry (GC/MS) was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a cold on-column injector and a capillary column (50 m × 0.32 mm i.d., HP-1) directly coupled to a mass spectrometer and integrated data system (70-250 VG Analytical and VG Autospec, Fisons Instruments). Ionization was by electron impact at 70 eV and 230 °C. The GC oven temperature was maintained at 30 °C for 5 min and then programmed at 5 °C/min to 250 °C. The carrier gas was helium. Tentative identifications made by GC/MS were confirmed by comparison of the mass spectral data with those of authentic samples and by peak enhancement when co-injected with authentic compounds using GC. All solvents were dried using anhydrous magnesium sulfate and were evaporated using a Büchi rotary evaporator. Solvents and reagents were used as supplied except when specified.

Raw Materials. Seeds of the summer cypress, *K. scoparia*, were produced by Colegrave Seeds Limited, West Adderbury, Banbury, Oxon., U.K., by cultivation under glass using standard methodology. Seed oil was extracted by English Hop Products Limited, Paddock Wood, Tonbridge, U.K. Carbon dioxide was employed as extractant (Laws et al., 1977), and the *K. scoparia* seeds (480 g) were extracted over a period of 7 h at 10 °C, 67 bar, and a flow rate of 6.5 kg of CO_2/h to give seed oil as a brown gum (39.9 g).

Chemical Conversion. *Synthesis of (5RS,6SR)-6-Hydroxy-5-hexadecanolide: Route A. (Z)-5-Hexadecenoic Acid (**3**).* To a solution of *K. scoparia* seed oil (3.0 g) containing glycerides of (*Z*)-5-hexadecenoic acid (**2**) in hexane (80 mL) was added dilute sodium hydroxide (2 M, 100 mL), and the mixture was heated under reflux overnight (18 h). The cooled solution was then washed with hexane (100 mL), and the aqueous phase was separated, cooled (ice bath), and then acidified using hydrochloric acid (6 M). Following extraction with ethyl acetate (3 × 100 mL), the combined organic layers were dried and evaporated under reduced pressure to give a crude yellow-green material (0.65 g). The presence of (*Z*)-5-hexadecenoic acid (**3**) (4.25% w/w) was confirmed by treatment of the material with boron trifluoride/methanol (Metcalfe and Schmitz, 1961) and location of the methyl ester by GC/MS analysis: *m*/*z* 268 (M^+, 20%), 236 (16), 194 (20), 152 (13), 110 (8), 96 (55), 74 (100), 67 (55), 55 (74), 43 (60), and 41 (73).

Methyl (Z)-5,6-Epoxyhexadecanoate. Methyl (*Z*)-5,6-hexadecanoate (**5**) (0.100 g, 0.0003 mol), obtained from a boron trifluoride/methanol esterification of **3**, was dissolved in hexane (20 mL) and introduced into a 100 mL round-bottom flask fitted with a magnetic bar and stirrer. To this solution was added with stirring over a period of 10 min a 20 mL solution of hexane containing *m*-chloroperbenzoic acid (0.5 g, 0.0003 mol). The combined solution was stirred at room temperature for a period of 24 h. A white precipitate was observed, and at the end of this period the precipitate was filtered and the filtrate washed with a solution of sodium sulfite (10%), followed by tests with starch iodide papers until the organic layer was observed to be free of oxidized substances. The organic layer was later washed with a (3 × 25 mL) solution of sodium bicarbonate (5%) and water (3 × 25 mL), dried over anhydrous magnesium sulfate, and filtered, and the solvent was removed using a rotary evaporator to leave behind a yellow oily product (0.065 g) containing the desired product: *m*/*z* 284 (M^+, 2), 225

(8), 183 (60), 143 (65), 141 (28), 113 (70), 99 (80), 83 (70), 71 (85), 59 (100), 55 (72), 43 (80).

*(5RS,6SR)-Dihydroxyhexadecanoic Acid (**4**).* To a solution of the crude yellow-green material (0.5 g) containing (*Z*)-5-hexadecenoic acid (**3**) in acetone (60 mL) was added a solution of *tert*-butylhydroperoxide (2 mL, 0.014 mol) in methanol (50%), followed by tetraethylammonium acetate·$4H_2O$ (0.2 g, 0.007 mol). The solution was allowed to stir at ambient temperature for 0.75 h and then cooled (ice bath) and treated with osmium tetraoxide (0.002 g, 0.00001 mol). After 1 h, the mixture was stirred at ambient temperature overnight (18 h) and then recooled (ice bath). Ethyl acetate (20 mL) and dilute sodium bisulfite solution (10%, 10 mL) were then added, the mixture was then stirred at ambient temperature for 1 h, and the resultant golden brown reaction was filtered with suction. The combined filtrate and washings were dried and evaporated to give an oil (0.38 g), which was utilized directly in the cyclization step.

*Synthesis of (5RS,6SR)-6-Hydroxy-5-hexadecanolide: Route B. Methyl (Z)-5-Hexadecenoate (**5**).* A solution of *K. scoparia* seed oil (1.0 g) in boron trifluoride/methanol (50%, 30 mL) was refluxed for 2 h, and the cooled reaction mixture was diluted with distilled water (100 mL) and then extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with dilute sodium bicarbonate solution (10%, 40 mL) and then dried and evaporated under reduced pressure to give a dark green oil (0.68 g). Dissolution of the oil in ethyl acetate (20 mL), followed by treatment with decolorizing charcoal and removal of the solvent, yielded a yellow, sweet-smelling oil (0.61 g). The presence of methyl (*Z*)-5-hexadecenoate (**5**) (4.5% w/w) in the oil was confirmed by GC/MS analysis: *m*/*z* 268 (M^+, 20%), 236 (16), 194 (20), 152 (13), 110 (18), 96 (50), 74 (100), 67 (52), 55 (71), 43 (62), 41 (86).

*Methyl (5RS,6SR)-5,6-Dihydroxyhexadecanoate (**6**).* To a solution of the yellow oil (0.32 g) containing methyl (*Z*)-5-hexadecenoate (**5**) in acetone (40 mL) was added a solution of *tert*-butylhydroperoxide (2 mL, 0.014 mol) in methanol (50%), followed by tetraethylammonium acetate·$4H_2O$ (0.2 g, 0.0007 mol). The solution was allowed to stir at ambient temperature for 0.75 h and then cooled (ice bath) and treated with osmium tetraoxide (0.002 g, 0.00001 mol). After 1 h, the mixture was allowed to stir at ambient temperature overnight (18 h) and then recooled (ice bath). Ethyl acetate (20 mL) and dilute sodium bisulfite solution (10%, 10 mL) were then added, the mixture was then allowed to stir at ambient temperature for 1 h, and the resultant mixture was filtered with suction. The combined filtrate and washings were dried and evaporated to give an oil (0.28 g), which was used directly in the cyclization step.

*Synthesis of (5RS,6SR)-6-Hydroxy-5-hexadecanolide: Route C (5RS,6SR)-5,6-Dihydroxyhexadecanoic Acid Triglyceride (**7**).* To a solution of *K. scoparia* seed oil (1.0 g) in acetone (40 mL) was added a solution of *tert*-butylhydroperoxide (2 mL, 1.4 mmol) in methanol (50%), followed by tetraethylammonium acetate·$4H_2O$ (200 mg, 0.7 mmol). The mixture was allowed to stir at ambient temperature for 0.75 h and then cooled (ice bath) and treated with osmium tetraoxide (2 mg, 0. 01 mmol). After 1 h, the mixture was allowed to stir at ambient temperature overnight (18 h) and then recooled (ice bath). Ethyl acetate (20 mL) and dilute sodium bisulfite solution (10%, 10 mL) were then added, and the mixture was then allowed to stir at ambient temperature for 1 h. Using the same workup procedure as in **6** above gave a yellow oil (0.87 g), which was used directly in the cyclization step.

*(5RS,6SR)-6-Hydroxy-5-hexadecanolide (**8**), General Procedure.* To a solution of the crude material containing either *(5RS,6SR)*-5,6-dihydroxyhexadecanoic acid (**4**), methyl *(5RS,6SR)*-5,6-dihydroxyhexadecanoate (**6**), or *(5RS,6SR)*-5,6-dihydroxyhexadecanoic acid triglyceride (**7**) in dry benzene (60 mL) was added *p*-toluenesulfonic acid (0.03 g, 0.0002 mol), and the mixture was allowed to stir under reflux for 2.5 h. After cooling to ambient temperature, the solvent was removed under reduced pressure and the dark brown oily residue taken up in ethyl acetate (30 mL). The organic layer was washed with saturated sodium bicarbonate solution (2 × 25 mL) and brine (20 mL) and then dried and evaporated to yield a viscous oil (0.30 g). A sample of the oil (0.1 g) was washed with hexane (3 × 15 mL), and the combined washings were evaporated under reduced pressure to give a dark oil. GC/MS analysis of the oil, followed by peak enhancement with an authentic sample [prepared by sodium borohydride deacetylation of an authentic sample of the oviposition pheromone, (5*R*,6*S*)-6-acetoxy-5-hexadecanolide] confirmed the presence of (5*RS*,6*SR*)-6-hydroxy-5-hexadecanolide (**8**) as a major component of the oil: ^{13}C NMR ($CDCl_3$) δ 14.1, 18.5, 21.5, 25.6, 29.7, 31.9, 71.6, 83.7, 170.8; *m*/*z* 270 (M^+, 0.04), 252 (0.7), 142 (30), 100 (100), 99 (85), 71 (36).

Using crude material (0.38 g) containing *(5RS,6SR)*-5,6-dihydroxyhexadecanoic acid (**4**) (prepared via route A) and methyl *(5RS,6SR)*-5,6-dihydroxyhexadecanoate (**6**) (prepared via route B), 0.22 g of dark oil was recovered, containing in each case 66 mg (30% w/w) of (**8**). Using crude material (0.47 g) containing *(5RS,6SR)*-5,6-dihydroxyhexadecanoic acid triglyceride (**7**) (prepared via route C), 0.165 g of dark oil was recovered, containing 74 mg (45% w/w) of **8**.

*(5RS,6SR)-6-Acetoxy-5-hexadecanolide ((**1a**) and (**1b**)).* To a solution of crude material (0.5 g) containing (5*RS*,6*SR*)-6-hydroxy-5-hexadecanolide (**8**) in dry pyridine (15 mL) was added acetic anhydride (0.5 mL, 0.0004 mol), and the mixture was allowed to stir at ambient temperature overnight (18 h). Following removal of the solvent under reduced pressure, the residue was taken up in ethyl acetate (15 mL) and then washed with dilute hydrochloric acid (3 M, 2 × 20 mL) and saturated sodium bicarbonate solution (15 mL) and brine (10 mL). The organic layer was dried and evaporated under reduced pressure to yield a yellow oil (0.35 g). GC/MS analysis of the oil showed the presence of *erythro* and *threo* diastereoisomers of 6-acetoxy-5-hexadecanolide (**1**): *m*/*z* 312 (M^+, 2%), 142 (20), 100 (52), 99 (100), 71 (28), 55 (33), 43 (85), 41 (24); ^1H NMR δ 0.88 (3H, t, CH_3), 1.25 [16H, m, $(CH_2)_8$], 1.60 (2H, m, CH_2), 1.71 (2H, m, CH_2), 2.0 (2H, m, CH_2), 2.08 (3H, s, CH_3), 2.53 (2H, m, CH_2), 4.30 (1H, m, CH), 4.98 (1H, m, CH); ^{13}C NMR δ 14.1, 18.2, 21.0, 22.7, 23.5, 25.1, 29.3−29.8 (seven signals), 31.9, 74.3, 80.5, 170.6, 170.8. Using an internal standard (tetracosane, $C_{24}H_{50}$), the amount of natural pheromone (i.e., the 5*R*,6*S* isomer) in the oil using (5*RS*,6*SR*)-6-hydroxy-5-hexadecanolide prepared following routes A, B, and C was estimated by GC analysis to be approximately 8, 12, and 16.5% (w/w), respectively.

Oviposition Bioassays. Cage bioassays with *Cx. quinquefasciatus* followed the method of Mordue (Luntz) et al. (1992). Test bowls contained either distilled water (100 mL) plus the test material (3 μL of either plant oil containing the oviposition pheromone or authentic oviposition pheromone). Materials were tested at a dose of 3 μg of pheromone per experiment. Control bowls contained distilled water (100 mL) plus hexane (3 μL). The number of egg rafts in the test bowls was recorded at the end of each experiment and converted to percentages of the total number of rafts in both bowls (control and test) for each cage, with differences between the test and control trials being determined by Student's *t* test.

RESULTS AND DISCUSSION

Prior to the planned synthesis of the oviposition pheromone **1a** from (*Z*)-5-hexadeconoic acid, the first step was to confirm that *K. scoparia* seed oil contained triglycerides of (*Z*)-5-hexadecenoic acid (**2**). The monounsaturated esters in the epoxidized seed oil after treatment with *m*-chloroperbenzoic acid and analysis of the resulting oil by GC/MS confirmed the presence of characteristic mass fragment ions for the epoxide of the 5-hexadecenoic acid methyl ester.

Having confirmed the presence of the (*Z*)-5-hexadecenoic acid, pheromone production was undertaken using three approaches depending upon the stage at which triglyceride cleavage was performed (see Scheme 1). The synthetic routes A and B involved initial

cleavage to give oils containing either the (*Z*)-5-hexadecenoic acid (**3**) or its methyl ester **5**, respectively, for further manipulation (Scheme 1, routes A and B). Ester preparation was accomplished via direct treatment of the triglycerides with boron trifluoride/methanol (Metcalfe and Schmitz, 1961). The oils containing **3** or **5** were treated with a catalytic amount of osmium tetraoxide (OsO_4) in the presence of *tert*-butylhydroperoxide (*t*-BOOH) (Akashi et al., 1978), to give oils containing (5*RS*,6*SR*)-dihydroxyhexadecanoic acid (**4**) or its methyl ester **6**, respectively. In general, catalytic osmylation of olefins in the presence of peroxides is a very effective method for the synthesis of 1,2-dihydroxy compounds. The reaction of (*Z*)-hexadecenoic acid (**3**) with a catalytic amount of osmium tetraoxide leads exclusively to the *erythro*-dihydroxyhexadecanoic acid (**4**) or its methyl ester **6** as a result of the formation of an intermediate osmiate ester, which when hydrolyzed gives exclusively the *cis*-dihydroxy product. The tetraethylammonium salt is used to regenerate the OsO_4, allowing glycolization to proceed and give the desired stereoisomeric products, **4** and **6**, in their enantiomeric *erythro* forms. Subsequent cyclization and acetylation on either material gave crude materials, which were shown by GC/MS to contain the 6-acetoxy-5-hexadecanolide. Peak enhancement using an authentic sample showed that mainly the *erythro* (5*RS*,6*SR*) isomers of the pheromone **1a** and **1b** were in the material, with little sign of the *threo* (5*RS*,6*RS*) isomers. Using an internal alkane standard (tetracosane, $C_{24}H_{50}$) for quantification, the materials were shown to contain approximately 16% (route A) and 24% (route B) (w/w) of the pheromone and its enantiomer. The other approach to the pheromone (Scheme 1, route C) involved direct treatment of the triglycerides in the seed oil with a catalytic amount of osmium tetraoxide in *tert*-butylhydroperoxide to obtain the dihydroxytriglyceride **7**. Cyclization and acetylation, as above, afforded a material containing the desired 6-acetoxy-5-hexadecanolide as confirmed by GC/MS. Peak enhancement using an authentic sample again showed the major presence of the *erythro* (5*RS*,6*SR*) isomers **1a** and **1b** and little sign of the *threo* isomers (Scheme 1, route C). The crude material was shown by the internal standard method to contain ~33% (w/w) of the pheromone and its enantiomer. Other identified extraneous plant materials and byproducts, which were carried over from the successive chemical conversion of *K. scoparia* seed oil to the crude reaction product containing the active pheromone 6-acetoxy-5-hexadecanolide, included hexadecanoic acid, octadecanoic acid, and 6-acetoxy-5-octadecanolide in varying amounts and quantities.

Because of the shorter route and greater yield of the oviposition pheromone **1a** in the crude material, the sample prepared via route C was used for subsequent bioassays. The behavioral activity of the crude material containing **1a** was assessed using standard oviposition cage bioassays for *Cx. quinquefasciatus* [Mordue (Luntz) et al., 1992]. With a dose of 3 μg of pheromone, egg laying was increased (Figure 1) (response = 69.7 ± 7.2%, $n = 8$, $P = 0.017$). The response was almost identical to that of an authentic sample of the pheromone at the same concentration (response = 72.9 ± 5.2%, $n = 8$, $P = 0.008$). Thus, as for the synthetic material (Dawson et al., 1990), the presence of nonbiologically active isomers in the oil does not hinder the bioactivity of the pheromone and, more significantly, neither does the presence of extraneous plant material.

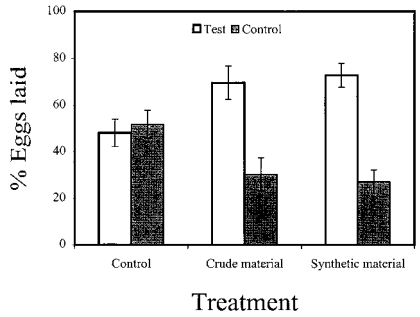


Figure 1. Oviposition behavior of gravid female *Cx. quinquefasciatus* in the presence of crude material (prepared using route C) containing 3 μg of oviposition pheromone ($n = 8$) and synthetic oviposition pheromone ($n = 8$). Vertical lines = ±SE.

On the basis of the yields obtained here, that is, 0.04 g of oviposition pheromone/g of *K. scoparia* seed oil, the estimated recurring cost of synthesizing the oviposition pheromone from seed oil ($3.00/g) compares favorably with that from conventional synthetic materials [e.g., $15.00/g using the route in Dawson et al. (1990)]. Furthermore, the high seed yields (11000 kg/ha) suggest that the pheromone yield would be in excess of 3 kg/ha. Thus, production of the pheromone via a renewable plant resource, which grows in developing countries, represents an important development in sustainable, cheap, and efficient production of this semiochemical. The ability of developing countries to grow *K. scoparia* as a crop for the production of the materials required for pheromone synthesis would provide the basis for the development of *Cx. quinquefasciatus* control strategies in resource-poor regions of the world. This would most likely involve a "lure-and-kill" approach, whereby the oviposition pheromone is used along with oviposition site derived attractants, for example, skatole (3-methylindole), to attract gravid females to sites containing either a highly selective larvicide or a larvae specific pathogen (Pickett and Woodcock, 1996).

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