erythro-6-Acetoxy-5-hexadecanolide, the Major Component of a Mosquito Oviposition Attractant Pheromone

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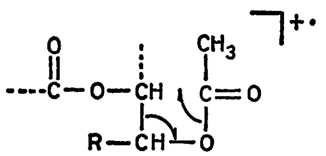
The major component of the oviposition attractant pheromone from the apical droplet of eggs of the mosquito *Culex pipiens fatigans* is shown by g.l.c.–mass spectrometry, microchemical methods, and synthesis to be *erythro*-6-acetoxy-5-hexadecanolide (**1**); laboratory tests have demonstrated the activity of synthetic (**1**).

The mosquito *Culex pipiens fatigans* (= *quinquefasciatus*) Wiedemann is distributed worldwide and in hot climates can be a vector for filarial diseases such as elephantiasis. Egg laying is influenced by a pheromone[1] which if identified could lead to an effective method of control. Eggs (20—150) are laid in rafts on stagnant water and apical droplets that form on the eggs release the volatile pheromone that attracts other gravid females to oviposit. Related mosquitoes less important as disease vectors, *Culex pipiens molestus* Forskal and *Culex tarsalis* Coquillett, are also attracted by the *Cx. p. fatigans* pheromone.[1] No volatile components from *Cx. p. fatigans* eggs have been described and only 1,3-diglycerides of mono- and dihydroxy-fatty acids, including *erythro*-5,6-dihydroxy-hexadecanoic acid, were identified in an active fraction obtained by t.l.c. of solvent washings of whole eggs of *Cx. tarsalis*.[2]

Apical droplets from eggs (5 raft equivalents) of *Cx. p. fatigans* were removed on fine glass rods and dissolved in hexane; examination of volatile components by g.l.c.–mass spectrometry† showed a major peak (R_t 64 min, relative ion current = 100) and others much smaller (e.g. R_t 53 min, 20; R_t 59 min, 25). The electron impact mass spectrum for the major component showed an ion at m/z 312 (0.1%) and an ion was present in the chemical ionisation spectrum at m/z 313 (100%) confirming M as 312. Accurate mass determination for significant ions at low resolution using the data system and with C_2I_4 as internal standard[3] indicated likely atomic compositions: m/z 312.2009, $C_{18}H_{32}O_4$ requires 312.2300; 252.1992 (3.3%), $C_{16}H_{28}O_2$ requires 252.2089; 142.0621 (35.9),

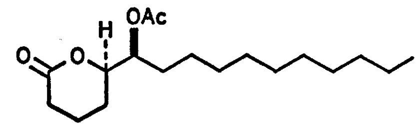
† Flexsil capillary column, 25 m × 0.2 mm, OV101, 50 °C (10 min), 4 °C/min to 200 °C, He flow 1 ml/min, directly coupled to mass spectrometer; electron impact 70 eV, 200 °C or chemical ionisation, isobutane (VG Micromass 70-70F + data system).



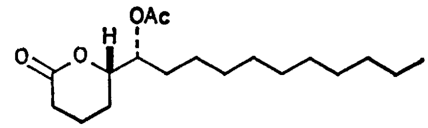
(A)

$C_7H_{10}O_3$ requires 142.0630; 100.0474 (49.4), $C_5H_8O_2$ requires 100.0524; 99.0432 (100), $C_5H_7O_2$ requires 99.0446. These suggested that the compound was an acetate (*m/z* 252, $M^+ - CH_3CO_2H$) and either a δ-lactone or a methyl γ-lactone (*m/z* 99, $O{:}CC_4H_7{:}O^+$).[4] The ion at *m/z* 142 containing 3 oxygen atoms could only arise in an unusual shift of CH_3CO from a neighbouring group on to the lactone ring [structure (A)]. α-Glycol diesters provide a precedent for such behaviour, the neutral fragments being expelled as aldehydes or ketones.[5]

T.l.c. (silica gel 60, 0.25 mm, with ether as eluant) of the apical droplet extract from 50 egg rafts gave a series of spots detectable with iodine vapour. Material from the region at R_f 0.39 (17 μg), containing the major volatile and the lowest R_t component, was treated with various reagents and analysed by g.l.c.–mass spectrometry. Hydrolysis (2 M NaOH) followed by acidification (conc. HCl) gave the hydroxy-lactone, R_t 59 min, *m/z* 270 (M^+, 0.05%), 252 ($M^+ - H_2O$, 0.5), 100 ($O{:}CC_4H_8O^+$, 100), and 99 ($O{:}CC_4H_7{:}O^+$, 25) which with *O,N*-bistrimethylsilylacetamide was converted into the trimethylsilyl ether, R_t 58 min, *m/z* 342 (M^+, 2.7%), 327 ($M^+ - CH_3$, 2.7), 243 ($M^+ - 99$, *i.e.* scission α to ether, 54.6), and 172 ($O{:}CC_4H_7SiMe_3O^+$, 59.1) and on acetylation (acetic anhydride/pyridine) gave the original compound. Reduction with the $NaH_2(MeOC_2H_4O)_2Al$ gave 1,5,6-trihydroxyhexadecane, R_t 65 min, *m/z* 238 ($M^+ - 2 \times H_2O$, 1.8%), 171 ($C_{11}H_{22}{:}O^+H$, 2.0), and 103 ($HOC_5H_9{:}OH^+$, 56.8), and thence the triacetate, R_t 90 min, *m/z* 341 ($M^+ - CH_3CO_2$, 5.0%), 280 ($M^+ - 2 \times CH_3CO_2H$, 2.5), and 187 ($CH_3CO_2C_5H_9{:}O^+CO.CH_3$, 30·4). Because the mass spectrum of the compound $C_{18}H_{32}O_4$ showed no evidence of chain branching, the likely structure was 6-acetoxy-5-hexadecanolide. Therefore the known *erythro*-5,6-dihydroxyhexadecanoic acid, prepared by *cis*-hydroxylation of (*Z*)-hexadec-5-enoic acid,[6] was treated with acetic anhydride in dry pyridine to give directly the racemic *erythro*-6-acetoxy-5-hexadecanolide (**1a** + **1b**) as a viscous oil, ^1H n.m.r. (Fourier transform, $CDCl_3$, Me_4Si ref.) δ 0.88 (t, CH_3), 1.26 (m, 8 × CH_2), 1.69—2.00 [m, C(3)H_2, C(4)H_2, and C(7)H_2], 2.08 (s, CH_3CO), 2.53 (m, CH_2CO), 4.35 [m, C(6)H or C(5)H], and 4.98 [m, C(5)H or C(6)H]. The mass spectrum was identical with that of the natural



(1a)



(1b)

product and a single peak was obtained when equal amounts of natural and synthetic material were coinjected on to the capillary column. T.l.c. of synthetic (**1**) gave a single spot with the expected R_f value. The *threo*-isomer obtained from (*E*)-hexadec-5-enoic acid chromatographed later, R_t 66 min, and gave more intense ions at *m/z* 269 (3.8%) and 252 (6.2%) than the *erythro*-isomer. Relative peak areas on g.l.c. indicated that each egg raft contained *ca.* 0.3 μg of compound (**1**). The presence of the C_{14} chain homologues in the derivatised samples and comparison with the spectrum of (**1**) showed the component with R_t 53 min to be 6-acetoxy-5-tetradecanolide. The component with R_t 59 min was identical with the product from hydrolysis–acidification of (**1**), 6-hydroxy-5-hexadecanolide.

In laboratory tests the synthetic *erythro*-6-acetoxy-5-hexadecanolide was as active an oviposition attractant for *Cx. p. fatigans* (5:1 ratio of egg rafts laid in treated against untreated dishes of water) as egg rafts containing an equivalent amount (25 egg rafts) of natural material.[1] Although these tests establish that pheromonal activity arises from compound (**1**), further studies which we are undertaking are necessary to establish feasibility of practical mosquito control.

Received, 5th October 1981; Com. 1163

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