established pheromone *E*8*E*10-12:OH. The occurrence of *E*9-12:OH in the blend seems at first unusual. A compound of matching retention range and amount was found in earlier analyses[5]. Biosynthesis of lepidopteran pheromones has been studied quite extensively, but no evidence for the pathways leading to the *E*-8,*E*-10 double bond system found in the codling moth has been presented. *E*9-12:OH could be the key compound for this pathway; in an analogous case, the 10,12-dienic system of bombykol was suggested to result from dehydrogenation of a Δ-11 monoene[17].

In our wind tunnel tests, 12:OH restores biological activity of overdoses of *E*8*E*10-12:OH, as observed for dodecyl acetate in *E. ambiguella*[15]. At low attractant doses it enhances biological activity, shifting the response curve by a factor of 4–20. The saturated alcohol could thus account for the 10-fold difference in attractiveness observed between *E*8*E*10-12:OH and an equivalent amount of female extract[7], but not for the factor of 1000 obtained when observing male excitation in another study[6]. This may indicate a role for some of the other female components.

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The identification of spiroacetals in the volatile secretions of two species of fruit fly *(Dacus dorsalis, Dacus cucurbitae)*

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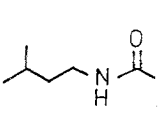
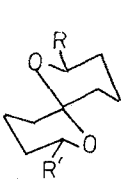
Summary. Aeration extracts from female *Dacus cucurbitae* and female *Dacus dorsalis* have been shown to contain a variety of 2,8-dialkyl-l, 7-diaxospiro [5.5] undecanes together with N-3-methylbutylacetamide.
Key words. Fruit fly; *Dacus dorsalis;* 2,8-dialkyl-l, 7-dioxaspiro [5.5] undecanes; N-3-methylbutyacetamide.

Both the melon fly, *Dacus cucurbitae* Coquillet, and the oriental fruit fly, *Dacus dorsalis* Hendel, are distributed throughout East Africa, India, Southeast Asia and Hawaii[1], and are serious pests of melon and other Cucurbitacea. Control is mainly by the use of insecticides. A synthetic attractant for male *D. cucurbitae,* 4-(p-acetoxyphenyl)-2-butanone (cuelure) is used for monitoring populations[2], while methyleugenol is used as a synthetic attractant for *D. dorsalis*[3]. Previous work from this laboratory[4] has shown that the rectal gland secretion of male *D. cucurbitae* contains 2-methoxy-N-3-methylbutyl acetamide together with two other amides, three pyrazine derivatives and 2-ethoxybenzoic acid. This work has now been extended to investigate the volatile secretions produced by adult female flies, *D. cucurbitae* and *D. dorsalis.*

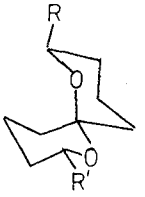
D. dorsalis and *D. cucurbitae* were taken from a laboratory culture originally obtained from USDA, Honolulu. The sexes were segregated within 1 day of emergence and kept at 25 ± 1 °C, 70 ± 5% relative humidity and a light intensity of circa 1000 lx maintained on a 13 h light: 11 h dark cycle. The volatile secretions produced by sexually mature (10-day-old) female fruit flies (50) were obtained by passing filtered air (25 ml min^{-1}) through a glass chamber containing live female flies, and absorbing the volatile compounds on an activated charcoal filter[5] (15 cm × 1 cm). After seven days the emitted volatiles were extracted from the charcoal filter by elution with dichloromethane (10 ml). The aeration extract was concentrated (100 µl) by distillation at atmospheric pressure and analyzed by gas chromatography (GC) and/or gas chromatography-mass spectrometry (GCMS). Typically, the extract was analyzed by GC (5% Carbowax 20M on 100–120 Diatomite AAW-DMCS, 5% OV101 on Chromosorb W-HP) and subsequently by GCMS (Kratos MS 30, EI 70 ev). The major component of the aeration extract from both species of fruit flies was N-3-methylbutyl acetamide (**1**) (1 µg/insect per day). The mass spectrum[6] and gas chromatographic properties were found to be identical to an authentic sample, prepared by unambiguous synthesis. The aeration extract from female *D. cucurbitae* gave two further major components which were identified as 2,8-dimethyl-1,7-dioxaspiro [5.5] undecanes on the basis of their mass spectra. Particularly characteristic were the fragment ions at m/z 112, 115[7]. (E, E)-2,8-Dimethyl-1,7-dioxaspiro [5.5] undecane (**2**) and (Z, E)-2,8-dimethyl-1,7-dioxaspiro [5.5] undecane (**3**) were synthesized by the method of Francke et al.[8]



(**1**)



(**2**) R=R^1=CH$_3$
(**4**) R=CH$_3$CH$_2$, R^1=CH$_3$
(**5**) R=CH$_3$CH$_2$CH$_2$, R^1=CH$_3$



(**3**) R=R^1=CH$_3$

and shown to be identical to the natural spiroacetals from *D. cucurbitae* by comparison of their GC retention time and mass spectra. The first derivative (**2**) (0.1 µg/insect per day) showed a molecular ion at m/z 184.1536 corresponding to $C_{11}H_{20}O_2$, m/z 184 (10%), 140 (25), 115 (95), 114 (30), 112 (100), 97 (50), 69 (40), 55 (40), 43 (40), whilst (**3**) (0.01 µg/insect per day) showed a molecular ion at m/z 184.1516 and ions at m/z 184 (5%), 140 (10), 125 (10), 115 (100), 114 (40), 112 (55), 97 (70), 69 (50), 55 (50).

Determination of relative configuration of spiroacetals has been carried out on the basis of solvent-depending shifts in ^{1}H-NMR and γ-effects in ^{13}C-NMR spectra[8]. Derivatives (**2**) and (**3**) have been reported previously as scent marks from the mandibular gland of the bees, *Andrena wilkella*[9], *A. ocreata* and *A. ovatula*[10] and may fulfil a similar role in fruit fly ecology. It would appear that these spiroacetals are not produced in the rectal gland of the female *D. cucurbitae*, because neither (**2**) nor (**3**) were identified by Baker et al.[4] as components of the rectal gland secretions.

From the aeration extract of female *D. dorsalis* three spiroacetals were identified. The two major components were (E,E)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**2**) (0.1 µg/insect per day) identified by comparison with a synthetic sample and the novel compound 8-ethyl-2-methyl-1,7-dioxaspiro[5.5]undecane (0.1 µg/insect per day). The latter showed characteristic fragment ions at m/z 112, 115 in the mass spectrum: m/z 198 (M^+, 10%), 169(20), 154(10), 140(20), 129(60), 115(100), 112(95), 97(45), 55(25). The molecular ion appeared at m/z 198 and the ion at m/z 169 (M − 29) arising from loss of ethyl indicated the alkyl substitution of the spiroacetal[7]. That 8-ethyl-2-methyl-1,7-dioxaspiro[5.5]undecane was the (E,E)-isomer (**4**) was indicated on the basis of the relatively short GC retention time. The minor spiroacetal component was identified as (E,E)-8-methyl-2-propyl-1,7-dioxaspiro[5.5]undecane (**5**) (0.01 µg/insect per day). This showed a mass spectrum and GC properties consistent with literature data[7,10].

Finally, a bishomologous series of ethyl esters of C_{12}, C_{14} and C_{16} saturated alkanoic acids was identified from *D. dorsalis*, together with the ethyl ester of the C_{16} monounsaturated alkanoic acid, all of which have been reported for *D. cucurbitae*[4] and *D. dorsalis*[11] from female rectal gland secretions. The role of the spiroacetals in the female fruit fly biology is as yet unknown, but is under investigation. It is of interest that only the female of the two *Dacus* species examined emits spiroacetals; the male aeration extracts have been examined but no spiroacetals were present.

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Structural characteristics of the carotenoids binding to the blue carotenoprotein from *Procambarus clarkii*

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Summary. A blue carotenoprotein from the crayfish *Procambarus clarkii* was extracted and purified. This carotenoprotein contains the carotenoid astaxanthin as a prosthetic group. In the present work we have identified by reconstitution, after removing the native carotenoid, some characteristics of the carotenoids that could bind to the apoprotein. The carotenoid must have two oxo groups at positions 4, 4′ and two hydroxyl groups at positions 3, 3′ the hexagonal or pentagonal end structure being indifferent. It has been proved that changes in the polyene chain structure such as triple bonds destroy this binding capacity.
Key words. Actinioerythrol; astaxanthin; carotenoprotein; *Procambarus clarkii;* reconstitution.

The blue carotenoproteins from crustaceans contain as prosthetic group the red carotenoid astaxanthin[1]. The interaction between the carotenoid and the colorless apoprotein changes the red color of the carotenoid into the blue color of the carotenoid-protein complex[2]. The carotenoid is easily removed by addition of organic solvents like acetone or DMF (dimethylformamide)[3,4], recovering its original red color. This change in color also occurs by denaturation of the protein by coccion, as is well known. Zagalsky has demonstrated[1] that after elimination of the astaxanthin from crustacyanin (a blue carotenoprotein from *Homarus gammarus*) the reconstitution of the carotenoprotein is only achieved if the carotenoids contain oxo groups at C-4. According to Remstrom et al.[5] the reconstitution of α-crustacyanin requires the presence of hydroxyl groups at C-3 in at least one of the rings of the carotenoid. In this work the reconstitution of the blue carotenoprotein from *Procambarus clarkii* with different kinds of carotenoids has been studied.

The blue carotenoprotein from the carapace of the crayfish *Procambarus clarkii* was extracted using the method of Quarmby et al.[6]. The crude extract was fractionated with ammonium sulfate, and the blue precipitate resuspended and dialyzed against phosphate buffer 20 mM pH 7.5, then adsorbed on to a DE-52 Whatman ionic exchange column. The blue fraction so obtained was chromatographed through a Sephacryl S-300 column, and eventually concentrated. This carotenoprotein solution showed only one band on electrophoresis by the method of Davis and Ornstein[7,8], indicating its purity. Reconstitution of the native carotenoprotein was achieved by the method of Britton et al.[4] using the following carotenoids: astaxanthin, 15,15′-didehydroastaxanthin, actinioerythrol, 3-hydroxyechinenone,