Chemistry of Fruit Flies: Composition of the Male Rectal Gland Secretions of some Species of South-East Asian Dacinae. Re-examination of *Dacus cucurbitae* (Melon Fly)

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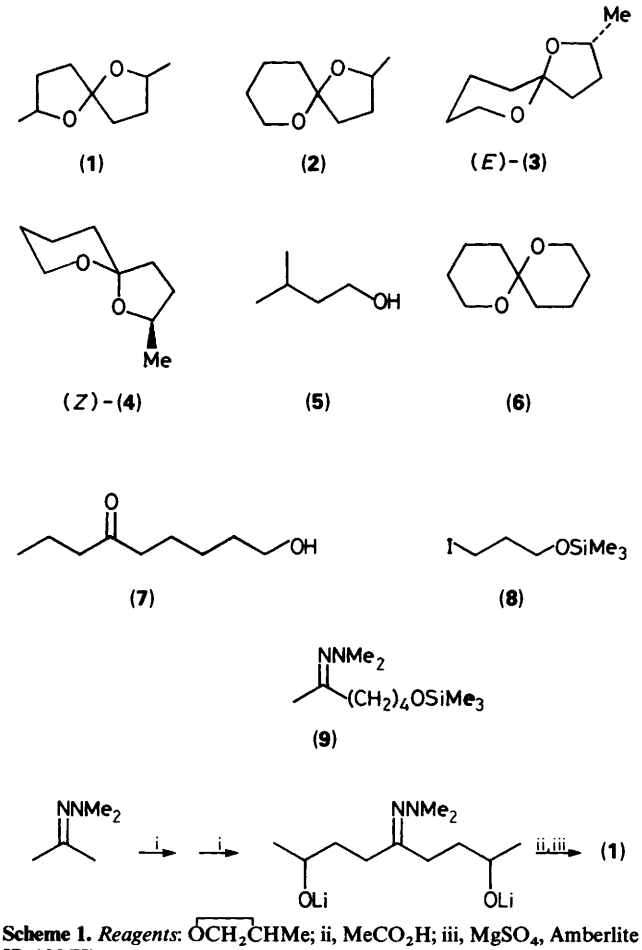
The major components of the rectal glandular secretions of male *Dacus* (*Bactrocera*) *umbrosus* (jack fruit fly), *D.* (*Bactrocera*) *nigrotibialis*, *D.* (*Bactrocera*) *albistrigatus*, *D.* (*Zeugodacus*) *tau* and *D.* (*Zeugodacus*) sp. (taxonomically similar to *D. tau*) have been identified. Alcohols, spiroacetals, and amides are the dominant components. The major component in the rectal gland secretion of *Dacus* (*Zeugodacus*) *cucurbitae* (melon fly) is shown to be ethyl 4-hydroxybenzoate and not 2-ethoxybenzoic acid.

In contrast to temperate species of fruit flies, which have a low capacity for dispersal and are monophagous or oligophagous, tropical Tephritidae have a high capacity for dispersal, and are usually polyphagous, with an expanding host range which exacerbates the problem of control.[1] Chemical cues regulate many phases of the biology of fruit flies, thus providing several points for chemical intervention and potential control.[1,2] With respect to mating, this is not simple, particularly among the tropical polyphagous species. In addition to pheromonal signals targeted at mate-searching females, there may be associated visual and/or other stimuli which should be considered in association with pheromone based methods for control or monitoring purposes. Nevertheless, the potential for pheromones to contribute to overall fruit fly monitoring and control in the Orient is substantial, and as a start in this general area, we have examined the rectal glandular components of a number of species that are of pest status in South East Asia.[3,4] (The male Tephritids store a pheromone in a reservoir and secrete it from a sac, both organs being located in the rectal region and appearing in the male about two days after the pupal–adult apolysis).[5,6]

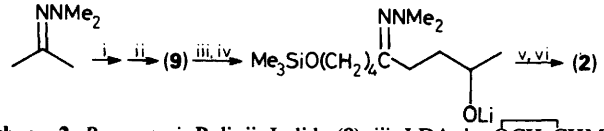
The species examined were *Dacus umbrosus* Fabricius, *D. tau* (Walker), *D. nigrotibialis* (Perkins), *D. albistrigatus* de Meijere and an undescribed *Dacus* (*Zeugodacus*) species. Clarification of the identity of the major component of the *D. cucurbitae* Coquillett secretion is also presented. The taxonomy of these species is presently being researched.[7]

Results and Discussion

Dacus umbrosus (*Jack-fruit Fly*).—The procedures for dissection and examination of glandular extracts have been described elsewhere.[8,9] *Dacus umbrosus* (jack-fruit fly) is widely distributed throughout Malaysia, Indonesia, Thailand, New Guinea, and the South-West Pacific areas,[4] and was reported as unique among Oriental and South Pacific *Dacus* species in being attracted to both male lures, methyl eugenol, and Cue-Lure.[10] The jack-fruit fly is, however, attracted to methyleugenol only, and infests various species of Artocarpus and has been bred from citrus and bread fruit.[11] GC/MS examination of the acetone extract of the rectal glandular secretion indicated that two isomeric compounds (26 and 18%) were the major components, with apparent M = 156, m/z 141 ($M - CH_3$) and characteristic spiroacetal fragmentations.[12] Consideration was given to systems (1) and (2) below, with the latter being confirmed by synthesis of both systems by sequential epoxide alkylation of acetone–*N*,*N*-dimethylhydrazone (Schemes 1, 2). To the best of our knowledge, no isomer of (1) has been identified as a natural product.



Scheme 1. *Reagents*: $\overline{OCH_2CHMe}$; ii, $MeCO_2H$; iii, $MgSO_4$, Amberlite IR-120(H).

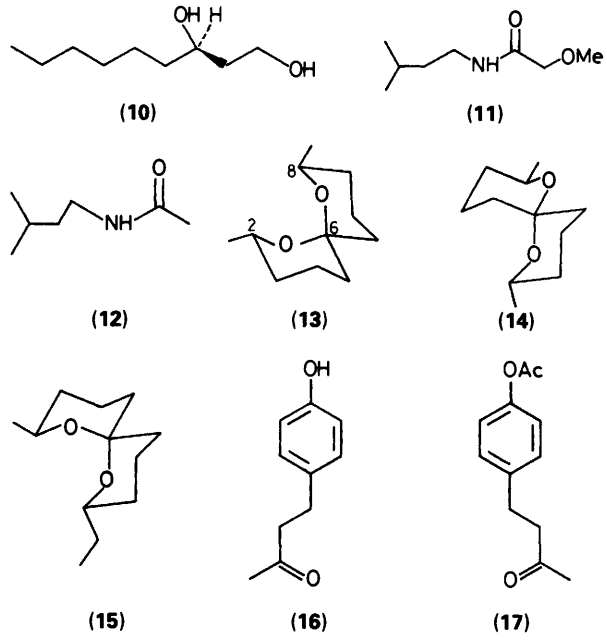


Scheme 2. *Reagents*: i, Buli; ii, Iodide (**8**); iii, LDA; iv, OCH_2CHMe; v, $MeCO_2H$; vi, $MgSO_4$ *etc.*

The mass spectra of the diastereoisomers of (**2**) were identical with those reported by Francke,[12b] who established the presence of (**2**) in workers of *Paravespula vulgaris*, the common wasp.[13] Chiral gas chromatography, using a camphoratonickel(II) chelate as stationary phase,[14] of synthetic racemic (**2**), and of the natural product showed the latter to consist primarily of one enantiomer of each of the (*E*) and (*Z*) diastereoisomers of the 2-methyl-1,6-dioxaspiro[4.5]decanes. Use of (*S*)-(−)-propylene oxide in Scheme 2 provided the (2*S*) enantiometers of the (*E*) and (*Z*) diastereoisomers of (**2**), and chiral gas chromatography established that the natural spiroacetals were predominantly (85% e.e.) the (2*R*,5*S*) (*E*) and (2*R*,5*R*) (*Z*) stereoisomers, (**3**) and (**4**) respectively, *i.e.* epimeric at the spiro centre. 3-Methylbutanol (**5**) (10%) and 1,7-dioxaspiro[5.5]undecane (**6**) (14%) were also present, along with 6-oxononan-1-ol (**7**) the latter being the major constituent in *D. occipitalis* (Bezzi).[15] The chirality of (**6**) has not been determined, but if results from examination of female *D. oleae*[16] and male *D. cacuminatus*[15] [in which (**6**) is the dominant glandular component] are a guide, (**6**) is almost certainly racemic. Several other unidentified components were present at a very low level in the extract.

Dacus tau.—*Dacus tau* is widespread in South East Asia and is a severe pest of cucurbits,[3,4] and an edible species of *Eugenia* (lilly-pilly) is an important host plant for this fly.[11] The rectal glandular secretion of *D. tau* (collected in Malaysia) consisted largely of one component (80%), exhibiting highest m/z = 113 (30%) and 75 (100%) in the EIMS. This component was shown to be nonane-1,3-diol by comparison with an authentic sample,[8] and further shown to be the *R* (−) enantiomer (**10**) by chiral gas chromatography, using a cyclodextrin-based phase. This diol, with the same configuration, is a minor (~10%) component in *D. cucumis*.[8] Two other components were the unusual methoxymethyl amide (**11**) (10%) which had been identified previously as a component of *D. cucurbitae* by Baker[17] and Lewis,[9] and the amide (**12**) (~2%) which is found also in *D. cucurbitae*,[9,17] *D. tryon*,[9,18] *D. aquilonis*,[9] and *D. neohumeralis*.[9,18] Five unidentified components, each *ca.* 1% were also present.

Dacus nigrotibialis.—*Dacus nigrotibialis* is located throughout Malaysia, Thailand, and Laos but this species has not been reared, and its host preferences are undefined,[11] although *Coffea robusta* has been suggested as a significant host.[19] The extract of this fly is rich in (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro-[5.5]undecane (85%) which was shown to be enantiomerically pure by chiral gas chromatography, with the (2*S*,6*R*,8*S*) configuration (**13**), as already established for this component in *D. cucumis*.[8] Of particular interest was the absence of the (*E*,*Z*)-diastereoisomer of this system (**14**), requiring enantiospecific hydroxylation in the formation of the notional keto-diol precursor. The unusual[20] even carbon-numbered spiroacetal (**15**), 2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane was also present (3%) and shown to be the (*E*,*E*) diastereoisomer as drawn in (**15**). This compound is present at a low level in other *Dacus* species, *e.g. D. halfordiae*,[8,15] *D. dorsalis*,[8] *D. latifrons*,[8] and *D. occipitalis*.[8] Enantiomers of (**15**) are currently being synthesized to permit chirality determination in the natural product. The 4-phenylbutanone derivative (**16**), structurally related to Cue-Lure (**17**), was a significant



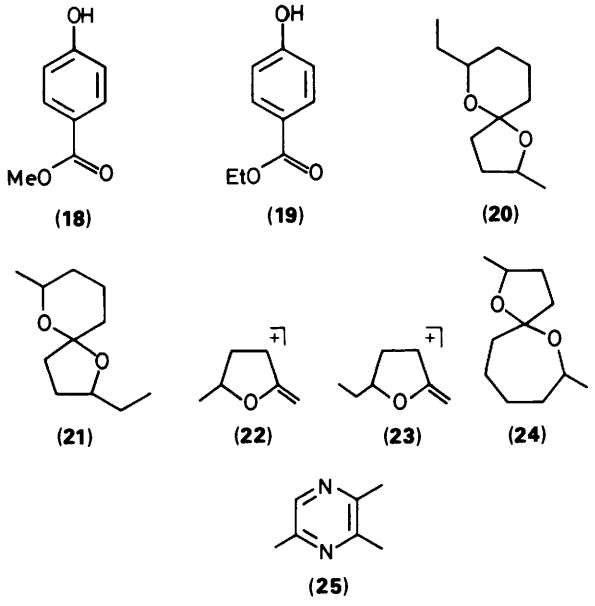
(**10**) (**11**) (**12**) (**13**) (**14**) (**15**) (**16**) (**17**)

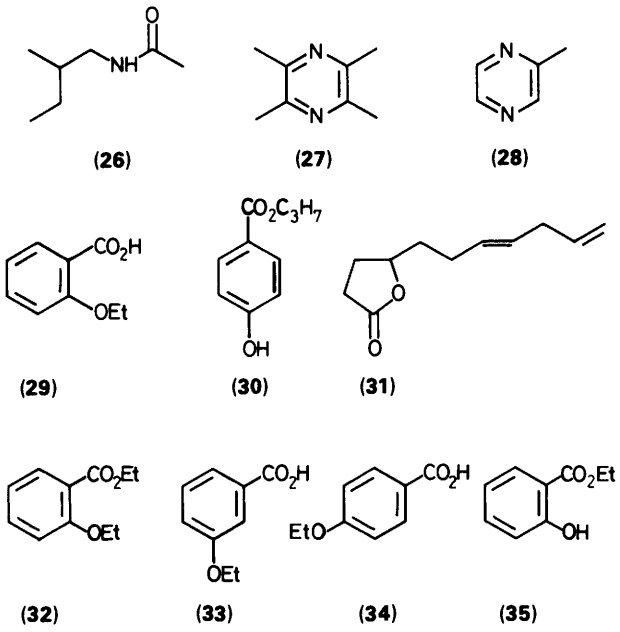
component (12%) but it is unclear whether this is a metabolite of (**17**), used as an attractant in the trapping of this species.

Dacus albistrigatus.—*Dacus albistrigatus* is distributed throughout Indonesia, Thailand, and Malaysia and is of minor pest status.[4] In Malaysia, the rain forest wild fruit *Terminalia catappa* is an important host, although in Indonesia specimens have been bred from guava.[11] (There is a possibility that the Indonesian variety is a sibling species.[11]) The acetone glandular extract was rich in methyl 4-hydroxybenzoate (**18**) identified by mass spectral comparisons with an authentic sample and fully discussed later. The (*E*,*E*)- and (*E*,*Z*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecanes (**13**) and (**14**), both of undetermined chirality were also present. [Note that the absolute configuration shown in (**13**) has been determined only for the (*E*,*E*) diastereoisomer in *D. nigrotibialus*.] The interesting point is that the *EE*:*EZ* ratio is 30:70, whereas in all other cases, the (*E*,*E*) diastereoisomer predominates, usually substantially.[21–23,8] This requires (on the assumption that a keto diol is involved biosynthetically) hydroxylation to occur predominantly with opposite chiralities at C_2 and C_8 along the undecane chain. However, there is little definite information on fruit-fly pheromone biosynthesis, and within a single species the 'pheromone blend' contains compounds that seem to require different biosynthetic routes.

Dacus (Zeugodacus) *sp.*—An undescribed, large *Dacus* (*Zeugodacus*) species was also collected in Malaysia and the major component (66%) of the acetone extract was ethyl 4-hydroxybenzoate (**19**) the mass spectral behaviour of which is discussed later. The pentane extract contained predominantly spiroacetals with (*E*,*E*) (**13**) (25%) (undetermined chirality) predominating along with the (*E*,*Z*) (**14**) (1%).

A significant component (8.6%) exhibited apparent M^+ = 184 (7%) with ions m/z = 169 ($M - CH_3$), 155 ($M - C_2H_5$), and other ions characteristic of the spiroacetal arrangements,[12] with the base peak m/z = 101. This component was suspected to be a diastereoisomer of 7-ethyl-2-methyl-1,6-dioxaspiro-[4.5]decane (**20**) and this was confirmed by the identity of the mass spectrum with the reported one,[12] and also with that of an independently synthesised sample.[24] The spiroacetal (**20**) is distinguished from its structural isomer 2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane (**21**) by the presence of the ion at m/z





98, whereas (**21**) exhibits an ion at *m/z* 112. These ions are considered to be (**22**) and (**23**) respectively.[12] Only one diastereoisomer of (**20**) was detected. A very minor component (~1%), while apparently lacking a molecular ion in the low resolution mass spectrum, exhibited a fragmentation pattern again strongly suggestive of a spiroacetal structure.[12] Based on gas chromatographic behaviour etc, and an ion *m/z* 169, a molecular weight of 184 was considered likely, with *m/z* 169 being $M - CH_3$. Other features of the spectrum, *e.g.* unusually intense ions *m/z* 140 and 111 and a very weak or absent molecular ion, indicated spiroacetal (**24**), *i.e.* 2,7-dimethyl-1,6-dioxaspiro[4.6]undecane, and this was confirmed by essential identity with the spectrum displayed by Francke[25] and the listed spectrum of a sample prepared by Mori.[26] Diastereoisomers of (**24**) occur as minor components of the complex volatile secretion from the mandibular glands of *Andrena haemorrhoa* (Hym., Apoidea).[25] The pyrazine (**25**) (3%) (found also in *D. cucurbitae*)[9,17] occurred along with substantial levels of C_{16} and C_{18} fatty acids, as have been observed in other *Dacus* species.[15]

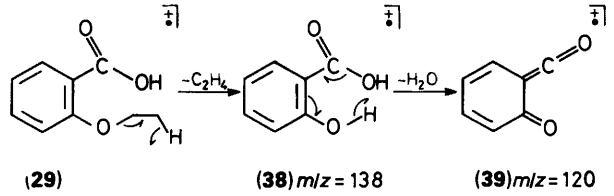
Dacus cucurbitae (*Melon fly*).—*Dacus cucurbitae*, the melon fly, is the major fruit-fly pest of melons and other cucurbits, and, world-wide, is one of the most active and destructive fruit-fly pests. The distribution range encompasses East Africa, South East Asia, Hawaii, and South Pacific Islands, but this species does not occur in Australia. Male melon flies are attracted to Cue-Lure (**17**) and thus monitoring of populations and infestations is possible. Several chemical studies of melon-fly populations have been published and another reported in thesis form. Baker, Herbert, and Lomer[17] studied the rectal glandular secretions of sexually mature male flies and showed that three amides were present, including the previously unreported 2-methoxy-*N*-3-methylbutylacetamide (**11**) along with (**12**) and (**26**). [(**11**) and (**12**) were shown above to be present in *D. tau.*] The pyrazines (**25**), (**27**), and (**28**) were reported, as well as ethyl esters of a number of fatty acids. However, the major component was concluded to be 2-ethoxybenzoic acid (**29**) (~4 μg/insect), on the basis of comparison of mass spectral data of the natural product and an authentic sample.

Most of the conclusions arrived at by Baker were confirmed by Lewis[9] in a study of wild male melon flies collected in the Phillipines. However, the amide (**26**) and the pyrazine (**28**) were not observed in this latter study, whereas a very minor component was concluded tentatively to be propyl 4-hydroxybenzoate (**30**). This raised the question whether the major component, concluded by Baker to be (**29**), was possibly ethyl 4-hydroxybenzoate (**19**). This matter was not pursued by Lewis.[9]

Studies of 'volatiles' emitted by melon flies have also been conducted. Prior to mating, male melon flies emit a 'smoke' like substance[27,28] from erectile ampoules on their rectum and analysis of this 'smoke' by Ohinata[27] indicated large quantities of trisodium phosphate, together with the hydrocarbons pentacosane, heptacosane, and nonacosane and the unusual lactone (**31**). Amides were not detected in the 'smoke'. Baker and Bacon[29] examined the volatile secretion produced by *female* melon flies ('aeration technique') and the major component was identified as the amide (**12**) with minor amounts of the spiroacetals (**13**) and (**14**) of undetermined chirality.

Our recent examination of the rectal glandular secretion of male melon flies requires that the constitution of the major component be revised from (**29**)[17] to (**19**), *i.e.* ethyl 4-hydroxybenzoate. This conclusion is based primarily on mass spectral fragmentation behaviour[30–32] and also GC retention comparisons. Authentic (**29**) was acquired by basic hydrolysis of (**32**) which resulted from treating salicylic acid with diethyl sulphate.

2-Ethoxybenzoic acid (**29**), was characterised from its ^1H and ^{13}C NMR spectra which were fully consistent with the structure and in agreement with those in a ^{13}C NMR data file.[33] In a similar way, the *meta* and *para* analogues of (**29**), *i.e.* (**33**) and (**34**) were obtained. The ethyl esters of *ortho*-, *meta*-, and *para*-hydroxybenzoic acids, *i.e.* (**35**), (**36**), and (**19**), were obtained by treating the appropriate hydroxybenzoic acid with



Scheme 3.

Table. Mass spectra (relative ion intensities) and relative retention times of hydroxybenzoic acid derivatives.

	(19)	(29)	(33)	(34)	(35)	(36)	(37)	(18)
m/z								
92	2.6	64	6.4	3.3	52.6	4.9	4.8	3.7
93	19.0	8.6	22.5	13.6	10.8	34.3	43.7	27.5
105(?)	—	4.8	—	—	—	—	—	—
120	—	*100*	4.7	—	*100.0*	—	—	—
121	*100.0*	14.0	65.3	*100*	27.7	*100*	*100*	*100*
138	23.0	2.5	*100.0*	63.7	2.5	25.5	—	—
151	2.5	17.9	—	—	—	5.5	—	—
152							47.6	33.0
166	18.8	14.5	54.9	44.3	34.0	34.5		

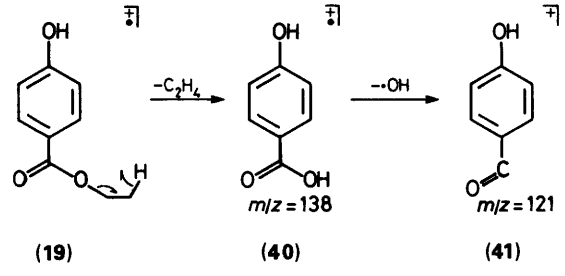
Relative retention time on SGE-BP5 column: 100 °C/min. Increase 16 °C/min.

1.71	1.76	1.55	1.65	1.00	1.62	1.44	1.53

ethanol and acid. The ^1H and ^{13}C NMR data were consistent with the structures and the ^{13}C spectra agreed with those in the ^{13}C NMR data file.[33,34]

The mass spectra of (**29**) and (**19**) are strikingly different. Compound (**29**) has its base peak at $m/z = 120$, which corresponds to $C_7H_4O_2$, *i.e.* (**39**) (Calc. for $C_7H_4O_2$: 120.0211. Observed 120.0210), which can be envisaged to arise by a double hydrogen transfer[30] (characteristic of *ortho* substituted systems) involving the ion (**38**) with $m/z = 138$ (3%) (Scheme 3).

In contrast, (**19**) exhibits the base peak at $m/z = 121$ which corresponds to (**41**). (Calc. for $C_7H_5O_2$: 121.0289. Observed 121.0284). Importantly, the mass spectrum of (**19**) lacks an ion at $m/z = 120$) (Scheme 4).



(**19**) (**40**) (**41**)

Scheme 4.

The mass spectra of (**19**) and its *meta* analogue (**36**) are similar in that both exhibit ions at $m/z = 138$, 121, and 93 but are, nevertheless, distinguishable on the basis of relative intensities (see Table). As noted above, ethyl salicylate (**35**) gives ion (**39**) (*i.e.*, $m/z = 120$) ('*ortho* effect') whereas the spectra of *meta*- and *para*-ethoxybenzoic acids (**33**) and (**34**), while somewhat similar to that of (**19**) (*i.e.* ions at 138, 121, and 93) are readily distinguished by relative intensities. The mass spectrum of the natural product is identical with that of (**19**).

This conclusion is supported by carefully measured retention times of non-derivatised samples on a non-polar capillary column (see Table). It should be noted that in *D. albistrigatus*, methyl 4-hydroxybenzoate (**18**) was a major component, and (**19**) is also present in the undescribed *Dacus* species alluded to above. There also seems little doubt that the minor component in *D. cucurbitae*, tentatively identified by Lewis[9] as propyl 4-hydroxybenzoate (**30**) is, in fact, (**30**),[30b] so that, in general, simple alkyl 4-hydroxybenzoates are the natural products, and these show some structural resemblance to the attractant Cue-Lure (**17**). However, care must be exercised with this analogy, as males of one of the major cucurbit-infesting flies in Southern Africa, *D. vertebratus* (taxonomically quite distinct from *D. cucurbitae*) are attracted[35] to methyl 4-hydroxybenzoate (**18**) (called Vert-lure), but not to Cue-Lure (**17**) or methyl eugenol (3,4-dimethoxyallylbenzene). In this region, Cue-Lure (**17**) does attract other economically important species, *e.g.* *D. bivittatus*, *D. punctatifrons*, and *D. frontalis*.[35]

Most studies of glandular secretions and volatile emissions of male Tephritid fruit-flies have concerned relatively few species, most of which are of commercial pest status.[36] Nevertheless, it is already clear there is considerable chemical diversity, and more varied biosynthetic routes appear to operate than for example in *Lepidopteran* species where the female utilises blends of similar compounds (to attract conspecific males) which are, in the main, of fatty acid origin. More extensive examination of fruit-fly species (some of which may not be serious pests) are warranted to define more precisely the chemistry and then biochemistry involved in the life-cycles of these insects, and possibly contribute also to taxonomic clarifications.[1,4] Chemical studies are continuing, and evaluations of the biological roles of certain components are being conducted.

Experimental

Spectra.—^1H NMR spectra were recorded at 400 MHz (FT mode) on a JEOL JNM-GX400 spectrometer, or in some cases at 60 MHz (CW mode) on a Varian EM360 spectrometer. Deuteriochloroform was employed as solvent, unless otherwise stated and chemical shifts (δ values) are relative to internal tetramethylsilane (0.0 ppm) or residual $CHCl_3$ (δ 7.24). ^{13}C NMR spectra were recorded at 100 MHz, utilising deuteriochloroform as solvent and chemical shifts are relative to the central component of the $CDCl_3$ triplet at 77.00 ppm, unless otherwise stated. Low resolution mass spectra refer to combined GC-MS measurements, recorded on a Finnegan Mat 1020 GC-MS system. High resolution mass spectra were recorded on a Kratos MS25-instrument. Optical rotations were recorded using a Perkin-Elmer 141 MC polarimeter. Preparative gas chromatography was performed using a Shimadzu gas chromatograph model GC-9A equipped with an OV101 column. Chiral gas chromatographic analyses were performed using either a 40 metre glass capillary column with hexakis (3-*O*-acetyl-2,6-di-*O*-pentyl)-α-cyclodextrin as the stationary phase or a 25 m bis(3-heptafluorobutyryl-(*R*)-camphorate)-nickel(II)/OV-1 fused silica column.

Isolation and Combined Gas Chromatography–Mass Spectrometry.—The flies were trapped in the field in the Gombok Rain Forest, Malaysis, using lure-baited traps. Cue-Lure attracted *Dacus tau*, *nigrotibialis*, *Zeugodacus* and methyleugenol attracted *D. umbrosus*. The *D. cucurbitae* were field collected (using Cue-Lure) in the Philippines. The design of the traps was such that the flies could not feed on the attractant. The flies were snap-frozen and stored in an ultra deep freeze (−80 °C) and then transported to Australia in dry ice and stored at −80 °C until dissection. The rectal glands were excised and extracted into acetone and in some cases, pentane.[8,9] Combined GC-MS examinations were performed using a Finnegan Mat 1020, employing a non-polar column.

Synthesis of Compounds

2,7-Dimethyl-1,6-dioxaspiro[4.4]*nonane* (**1**).—To a stirred solution of acetone-*N,N*-dimethylhydrazone[37] (1.95 g, 19.5 mmol) in dry THF (25 ml) (N_2 atmosphere; −78 °C) was added dropwise a solution of butyl-lithium in hexane (12.6 ml, 20.4 mmol). On completion of addition, the reaction mixture was stirred for 1 h, during which time a white solid formed.

Propylene oxide (1.2 g, 20.7 mmol) in THF (5 ml) was then added to the cooled (−78 °C) solution, after which the reaction mixture was slowly brought to room temperature (20 °C) and then stirred overnight. The reaction mixture was recooled to −78 °C and again treated with butyl-lithium (13.0 ml, 20.8 mmol) after which warming to room temperature occurred. After being stirred for 4 h at this temperature, the recooled solution (−78 °C) was treated dropwise with propylene oxide (1.25 g, 21.5 mmol) in THF (5 ml) and allowed to warm to 20 °C; it was then stirred overnight.

This mixture was neutralised with glacial acetic acid (2.4 g, 40 mmol) and filtered to remove the precipitated lithium acetate; the latter was then rinsed with dry THF. The filtrate, Amberlite IR-120(H) ion exchange resin (29 g), and anhydrous $MgSO_4$ (20 g) were stirred and refluxed for 5 h, the reaction being monitored by GLC. The reaction mixture was filtered and the THF removed (rotary evaporator) to yield an oil which was extracted into methylene dichloride. This organic phase was washed with saturated aqueous sodium carbonate and water, separated, dried ($MgSO_4$), and evaporated to yield a volatile oil (1.6 g; 52%). Final purification by preparative GC yielded the compound as a diastereoisomeric mixture (*EE*:*EZ*:*ZZ* = 30:50:20) (Found: C, 68.4; H, 10.55. $C_9H_{16}O_2$ requires C, 69.19; H, 10.32); δ_C(100 MHz) (*E*,*E*): 21.10, 32.17, 35.82, 73.87 or 74.04, and 114.91; (*E*,*Z*): 21.32, 22.75 or 22.72, 31.84, 32.64, 35.38, 36.85, 73.87 or 74.04, 75.77, and 114.76; (*Z*,*Z*): 22.72 or 22.75, 32.56, 36.62, 75.79, and 114.53; δ_H(400 MHz) (mixture of *E*,*E*, *E*,*Z*, and *Z*,*Z*): 1.16 (d, 6.0 Hz, CH_3, *E*,*E* 6 H or *E*,*Z* 3 H), 1.18 (d, 6.0 Hz, *E*,*E* 6 H or *E*,*Z* 3 H, CH_3), 1.25 (d, 6.0 Hz, *E*,*Z* 3 H, CH_3), 1.27 (d, 6.0 Hz, *Z*,*Z* 6 H, CH_3), 1.36–2.14 (m, 24 H, CH_2, *E*,*E*, *E*,*Z*, *Z*,*Z*), 4.0–4.34 (m, 6 H, CH–O, *E*,*E*, *E*,*Z*, *Z*,*Z*); *m*/*z* (*EZ*) 156 (M^+, 5.4%), 141 (15.8), 112 (33.1), 101 (100), 100 (20.1), 85 (39.5), 83 (42.9), 56 (36.8), and 55 (31.0); *m*/*z* (*ZZ*) 156 (M^+, 7.2%), 141 (16.2), 112 (34.3), 101 (100), 100 (20.9), 85 (44.3), 83 (43.9), 56 (42.7), and 55 (32.3); *m*/*z* (*EE*) 156 (M^+, 4.0%), 141 (13.2), 101 (100), 100 (19.4), 85 (35.6), 83 (43.9), 56 (37.4), and 55 (39.8) (Found: M^+, 156.1148. $C_9H_{16}O_2$ requires *M*, 156.1150).

1-*Iodo*-3-*trimethylsiloxypropane* (**8**).—To a stirred solution of 3-bromopropanol (7.0 g, 50 mmol) and 2,6-lutidine (5.89 g, 55 mmol) in carbon tetrachloride (90 ml) at 0 °C, was added chlorotrimethylsilane (7.0 ml, 55 mol). This solution was stirred at room temperature for 3 h and then filtered through basic alumina/Celite (1:1). Solvent was removed (rotary evaporator) and the resulting oil was distilled (60 °C/8 mmHg) to provide 1-bromo-3-trimethylsiloxypropane (6.73 g, 64%). This product was added dropwise to sodium iodide (5.26 g, 35 mmol) in dry acetone and the mixture stirred for 3 h after which time it was filtered (basic alumina) and then distilled (65 °C/5 mmHg) to give the title compound (**8**) (5.16 g, 62%), δ_C − 0.54, 3.33, 35.93, and 61.75; δ_H 0.08 (s, 9 H, $SiCH_3$), 1.95 (p, 6.3 Hz, 2 H, $CH_2CH_2CH_2$), 3.22 (t, 6.7 Hz, 2 H, CH_2I), and 3.59 (t, 5.9 Hz, 2 H, CH_2O).

6-*Trimethylsiloxyhexan*-2-*one Dimethylhydrazone* (**9**).—To the lithium derivative of acetone *N*,*N*-dimethylhydrazone[37] (1.0 g, 10 mmol) generated with butyl-lithium (4.4 ml, 11 mmol) in the manner described above, was added 1-iodo-3-trimethylsiloxypropane (**8**) (2.6 g, 10 mmol) in THF (5 ml), and the reaction mixture was allowed to warm to room temperature; it was then stirred overnight. The reaction mixture was then diluted with ether–hexane (1:1), filtered through basic alumina, and evaporated to provide an oil which was distilled (65 °C/0.3 mmHg) to give the title compound (1.54 g, 67%); δ_C (major isomer) −0.69, 16.17, 23.12, 32.02, 38.49, 46.78, 62.07, and 167.37; δ_H −0.04 (s, $SiCH_3$), 1.40 (m, 4 H), 1.80 (s, 3 H, CH_3), 2.06 (m, 2 H), 2.28 (s, 6 H, NMe_2), and 3.44 (m, 2 H); *m*/*z* 230 (M^+, 16.9), 186 (8.6), 172 (10.4), 171 (25.2), 147 (11.2), 129 (8.7), 113 (13.1), 103 (13.4), 100 (30.8), 98 (21.7), 83 (10.9), 75 (53.5), 73 (100), and 60 (61.6) (Found: M^+, 230.1822. $C_{11}H_{26}N_2OSi$ requires M^+, 230.1814).

2-*Methyl*-1,6-*dioxaspiro*[4.5]*decane* (**2**).—To a solution of lithium di-isopropylamide (prepared from butyl-lithium [2.5M solution; 2.2 ml, 5.5 mmol] and di-isopropylamine (0.5 g, 4.9 mmol) in dry THF (20 ml) (at −78 °C) with an argon atmosphere] was added 6-trimethylsiloxyhexan-2-one dimethylhydrazone (**9**) (0.7 g, 3 mmol) in THF (5 ml); the reaction mixture was then maintained at 0 °C for 3 h after which it was recooled to −78 °C. Propylene oxide (0.6 g, 10 mmol) was then added dropwise after which the mixture was allowed to slowly warm to room temperature when it was stirred overnight. Acetic acid (0.6 g, 10 mmol) was added to the mixture and the precipitated lithium acetate was removed by filtration through Supercell; the filtrate was then stirred and refluxed with anhydrous $MgSO_4$ (5 g) and Amberlite IR-120(H) (3.5 g) for 5 h.[38] This mixture was worked-up in the manner detailed previously and the resulting oil was distilled (120 °C/100 mmHg) to provide (**2**) (0.3 g, 64%) as a mixture of *E*- and *Z*-diastereoisomers (62:38); δ_C (*E*-isomer) 20.20, 21.16, 25.21, 31.31, 34.13, 37.66, 61.49, 73.92, and 105.75; (*Z*-isomer) 20.26, 23.07, 25.30, 31.62, 33.98, 38.90, 61.35, 76.65, and 105.55; δ_H 1.18 [d, 6.3 Hz, 3 H, CH_3(*E*)], 1.25 [d, 6.1 Hz, 3 H, CH_3(*Z*)], 1.3–2.1 (m, 20 H, ring CH_2), 3.52 (m, 2 H), 3.79 [t of d, 11.3, 3.0 Hz, 1 H, 7-H (axial) (*E*)], 3.86 [t of d, 11.5, 3.0 Hz, 1 H, 7-H axial (*Z*)], and 4.16 (m, 2 H); *m*/*z* (*E*-isomer) 156 (M^+, 5%), 141 (3.3), 128 (4.6), 112 (12.9), 111 (11.9), 101 (100), 100 (33.5), 98 (46.36), 83 (41.5), 56 (31.9), 55 (49.8), and 43 (38.9); *m*/*z* (*Z*-*isomer*) 156 (M^+, 6.2), 141 (3.6), 128 (4.8), 112 (10.3), 111 (10.7), 98 (41.7), 83 (39.8), 56 (31.6), 55 (50.1), and 43 (37) (Found: M^+, 156.1139. $C_9H_{16}O_2$ requires M^+, 156.1150).

(2S,5RS)-2-*Methyl*-1,6-*dioxaspiro*[4.5]*decane* (**3**) *and* (**4**).—The procedure employed was identical with that for the racemic material except that (*S*)-(−)-propylene oxide (Aldrich Chemical Co.) was used. The product exhibited spectroscopic properties identical with those obtained for the racemate and had $[\alpha]_D^{20}$ = −10.5° (*c* 3.3, methanol). Chiral gas chromatography established e.e. = 98% (lit.,[39] $[\alpha]_D^{20}$ = −10.2°. e.e. = 95.2%. *E*/*Z* = 65:35).

2-*Methoxy*-N-3-*methylbutylacetamide* (**11**).[17]—To a cooled solution (0 °C) of triethylamine (3.03 g, 30 mmol) in dry ether (30 ml) was added dropwise chloroacetyl chloride (2.82 g, 25 mmol) in dry ether (30 ml). A white precipitate formed. 3-Methylbutylamine (2.63 g, 30 mmol) in dry ether (30 ml) was added dropwise to the mixture, which was then warmed to 20 °C and stirred overnight. The reaction mixture was washed with water (100 ml) and separated, and the organic phase was dried ($MgSO_4$) and evaporated to provide 2-chloro-*N*-3-methylbutylacetamide (1.2 g, 30%); δ_C 22.23, 25.69, 37.81, 38.33, 42.43, and 169.94. This material (1.0 g, 6.1 mmol; crude) in dry ether (20 ml) was added dropwise to a stirred solution of sodium methoxide (from 0.5 g of sodium in dry methanol). The reaction was monitored by GC and after 2 days at *ca.* 20 °C the mixture was diluted with water and the methanol/ether removed (rotary evaporator). The residue was extracted with ether (2 × 50 ml) and the extract dried ($MgSO_4$) and evaporated to yield 2-methoxy-*N*-3-methylbutylacetamide (**11**) (0.5 g, 51%); δ_C 22.13, 25.53, 36.78, 38.16, 58.83, 71.74, and 169.06; δ_H: 0.78 (d, 6.7, 6 H, Pr^i), 1.28 (q, 7.0 Hz, 2 H, $CHCH_2CH_2$), 1.49 (m, 1 H), 3.17 (q, 6.5 Hz, 2 H, CH_2N), 3.27 (s, 3 H, OCH_3), 3.73 (s, 2 H, CH_2O), and 6.45 (br s, 1 H, NH); *m*/*z* 159 (M^+, 2.3), 129 (27.6), 114 (18.4), 103 (19.5), 102 (18.0), 73 (27.5), 71 (49.12), 55 (13.3), 45 (88.64), 44 (22.6), and 43 (100).

Methyl 3-Hydroxybenzoate (**37**).—This compound was prepared from 3-hydroxybenzoic acid and BF_3·MeOH (20%) in anhydrous methanol. It was recrystallised from benzene–hexane, m.p. 68 °C;[34] δ_C 52.48, 116.47, 120.61, 121.77, 129.76, 131.14, 156.21, and 167.89; δ_H 3.90 (s, 3 H, OMe), 7.09 (ddd, *J* 8, 2.5, 1.0, 4-H), 7.28 (s, OH), 7.28 (t, *J* 8, 5-H), 7.57 (dt, *J* 8, 1.4, 6-H), and 7.61 (m, br, H_2); *m/z* 152 (M^+, 47.6), 121 (100), 93 (43.7), 65 (30.7), and 39 (38.5).

Methyl 4-Hydroxybenzoate (**18**).—This compound was available as a commercial sample;[34] *m/z* 152 (M^+, 33.0), 121 (100), 93 (27.5), 65 (27.7), and 39 (26.4).

Ethyl 3- and 4-Hydroxybenzoates (**36**) *and* (**19**).—To either of the hydroxybenzoic acids (2.75, 20 mmol) in ethanol (50 ml) was added concentrated H_2SO_4 (2 drops) and the mixture was refluxed overnight. After neutralisation with aqueous $NaHCO_3$, the ethanol was removed (rotary evaporator) and on cooling, the ethyl esters crystallised from solution.

Ethyl 4-hydroxybenzoate (**19**). M.p. 116–117;[34] δ_C($CDCl_3$/DMSO) 13.01, 58.70, 114.10, 118.87, 130.06, 161.59, and 164.85; δ_H(60 MHz) ($CDCl_3$/DMSO) 1.4 (t, *J* 6, CH_3), 4.33 (q, *J* 6, CH_2O), 6.83 (d, *J* 8, 3-, 5-H), 7.87 (d, *J* 8, 2-, 6-H), 7.95 (s, OH); *m/z* 166 (M^+, 18.8), 138 (23.0), 121 (100), 93 (19.0), 65 (22.5), and 39 (21.2) (Found: 121.0284. $C_7H_5O_2$ requires 121.0289).

Ethyl 3-hydroxybenzoate (**36**). M.p. 72–73 °C;[34] δ_C 14.08, 61.45, 116.34, 120.43, 121.56, 129.60, 131.32, 156.14, and 167.37; δ_H(60 MHz) 1.4 (t, 6 Hz, CH_3), 4.36 (q, 6 Hz, CH_2O), and 6.95–7.66 (m, 4 H, aromatic H); 166 (M^+, 34.5), 138 (25.5), 121 (100), 93 (34.3), 65 (25.0), and 39 (34.5).

Ethyl 2-hydroxybenzoate (*ethyl salicylate*) (**35**). This compound was available as a commercial sample;[34] *m/z* 166 (M^+, 34.0), 138 (2.5), 121 (27.7), 120 (100), 93 (10.8), 92 (52.6), 65 (19.5), and 39 (24.9).

2,3- *and* 4-*Ethoxybenzoic Acids* (**29**), (**33**), *and* (**34**).—Each of the 2-, 3-, and 4-hydroxybenzoic acids (2.75 g, 20 mmol), NaOH (2.4 g, 60 mmol), diethyl sulphate (9.24, g, 60 mmol), and benzyltriethylammonium chloride (0.7 g) in a dichloromethane (50 ml)–water (50 ml) were stirred at 20 °C for 40 h. The organic layer was separated and the water layer extracted with dichloromethane (2 × 20 ml). The organic layers were then combined and evaporated and the residue was taken up in ether (50 ml). This solution was washed with aqueous ammonia (30 ml) and 10% aqueous NaOH, dried ($MgSO_4$), and evaporated.

The crude ethyl ethoxybenzoates were stirred with 10% aqueous NaOH for *ca.* 2 days after which time the basic solutions were washed with ether (2 × 50 ml) and acidified with HCl. The precipitated acids were collected by filtration although for 2-ethoxybenzoic acid (**29**) (m.p. 19 °C), the product was extracted into ether, and the extract dried ($MgSO_4$), and evaporated.

2-Ethoxybenzoic acid (**29**). M.p. 19 °C,[34] b.p. 170 °C/0.1 mmHg; δ_C 14.38, 65.77, 112.59, 118.98, 121.79, 133.28, 134.85, 157.41, and 165.96; δ_H(60 MHz) 1.53 (t, *J* 6 Hz, CH_3), 4.35 (q, *J* 6 Hz, CH_2O), 6.95–8.27 (m, 4 H, ArH), and 9.33 (br s, OH); *m/z* 166 (M^+, 14.5), 151 (17.9), 121 (14.0), 120 (100), 93 (8.6), 92 (64), 65 (15.3), 64 (16.1), 63 (12.1), and 39 (22.5).

3-Ethoxybenzoic acid (**33**). M.p. 137–138 °C;[34] δ_C($CDCl_3$/DMSO) 13.99, 62.80, 114.12, 118.78, 121.18, 128.55, 131.48, 158.06, and 167.50; δ_H 60 MHz ($CDCl_3$/DMSO) 1.27 (t, 6 Hz, CH_3), 3.93 (q, 6 Hz, CH_2O), 6.83–7.67 (m, ArH and OH); *m/z* 166 (M^+, 54.9), 138 (100), 121 (65.3), 93 (22.5), 65 (26.9), 64 (10.6), 53 (10.24), 43 (14.5), and 39 (25.1).

4-Ethoxybenzoic acid (**34**). M.p. 198–199 °C;[34] δ_C($CDCl_3$/DMSO) 13.84, 62.78, 113.12, 122.18, 130.83, 161.69, and 167.26; δ_H(60 MHz) ($CDCl_3$/DMSO) 1.30 (t, 6 Hz, CH_3), 4.06 (q, 6 Hz, CH_2O), 6.8 (d, 9 Hz, 3-, 5-H), 7.87 (d, 9 Hz, 2-, 6-H); *m/z* 166 (M^+, 44.2), 138 (63.7), 121 (100), 93 (13.6), 65 (21.1), and 39 (21.2).

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