Supplementary Material Available: Structural report for the (2,4-dinitrophenyl)hydrazone of **28b**, including anisotropic thermal parameters, a detailed description of data collection, structure solution, and refinement, and 200-MHz ^{1}H NMR spectra of the (2,4-dinitrophenyl)hydrazone of **28b** and of compounds **5**, **12**, **13**, **16** (R = Me), **18** (R = Me), **19** (R = H), **27** + **29** (R = Me, R′ = TBS), **27** (R = Me, R′ = COPh), **28** + **30**, **39–41**, **47**, **48**, **52**, **54**, **58**, **60**, **62**, and **64** (37 pages); tables of observed and calculated structure factors (11 pages). Ordering information is given on any current masthead page.

Chemistry of Fruit Flies. Composition of the Rectal Gland Secretion of (Male) *Dacus cucumis* (Cucumber Fly) and *Dacus halfordiae*. Characterization of (*Z*,*Z*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane

William Kitching,*[†] Judith A. Lewis,[†] Michael V. Perkins,[†] Richard Drew,[‡] Christopher J. Moore,[‡] Volker Schurig,[§] Wilfried A. König,[∥] and Wittko Francke[∥]

Department of Chemistry, University of Queensland, St. Lucia, Queensland, Australia 4067, Department of Primary Industries, Brisbane, Australia 4067, Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, D7400, Federal Republic of Germany, and Institut für Organische Chemie der Universität, Martin-Luther-King Platz 6, D-2000, Hamburg, Federal Republic of Germany

Received December 30, 1988

Combined gas chromatography–mass spectrometry and synthesis have established that the major components of the rectal gland secretion of adult male cucumber flies (*Dacus cucumis*) are the *E*,*E*, *E*,*Z*, and *Z*,*Z* diastereomers of 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, which have been synthesized utilizing organomercury chemistry from 1,10-undecadien-6-one. The thermodynamically least stable *Z*,*Z* diastereomer has been characterized for the first time, by ^{1}H and ^{13}C NMR measurements and mass spectrometry. Minor amounts of 3-hydroxy-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane and various derivatives of the 1,6-dioxaspiro[4.5]decane and 1,7-dioxaspiro[5.6]dodecane systems are also present. 1,3-Nonanediol occurs in significant amounts, and both enantiomers have been acquired by Sharpless asymmetric epoxidation of (*E*)-2-nonen-1-ol followed by Red-Al (Aldrich) reduction of the chiral epoxy alcohols. Chiral gas chromatographic analysis of the diols (as their cyclic carbonates) has demonstrated that the natural material is the *R*-(−) enantiomer. Natural (*E*,*Z*)-2,8-dimethyl-1,7-dioxaspiro-[5.5]undecane is shown to be racemic, whereas the more abundant *E*,*E* diastereomer is enantiomerically highly pure, possessing the 2*S*,6*R*,8*S* configuration. The major volatile component of the rectal gland secretion of male *Dacus halfordiae* (Tryon) is also (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, with no detectable levels of the *E*,*Z* or *Z*,*Z* isomers. Other spiroacetals present were 2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane and the unusual even carbon-numbered (*E*,*E*)-2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane. 6-Oxo-1-nonanol, diethyl succinate, and 2-methyl-6-pentyl-3,4-dihydro-2*H*-pyran were also identified.

Introduction

Fruit flies are destructive pests of horticulture in the tropical and temperate world and elaborate detection systems are employed for population monitoring and timing of eradication programs.[1] In recent years, more attention has been directed to the possible use of pheromone-based attractants, following the demonstration[2,3] that male tephritids store the pheromone in a reservoir and secrete it from a sac, both glands being located in the rectal region and appearing in the male (e.g., of Queensland fruit fly, *Dacus tryoni*) 2 days after the pupal–adult apolysis.[2] As the flies mature, droplets of yellowish material form.[2] In only one species, *D. oleae* (olive fly) does the female release the sac pheromone,[4] whereas male-produced pheromones have been identified in several species of tephritid fruit flies including *D. tryoni*,[5] *D. dorsalis*,[6] *D. cucurbitae*,[6] *Anastrepha suspensa*,[7] *A. ludens*,[8] and *Ceratitis capitata*.[9] These secretions are multicomponent in nature. What appear to be sex pheromones have also been found in the males of *Dirioxa pornia* (Walker),[10] *Rhagholitis pomonella* (Walsh),[11] and *R. cerasi L*,[12] but chemical information is lacking. The release of the pheromone is generally accompanied by courting behavior and the females may respond to other visual, auditory, or gustatory stimuli.[1]

Bellas and Fletcher[13] conducted the first chemical examination of the reservoir secretion of a Dacine species, and this work concerned *D. tryoni* (Froggatt) (Queensland fruit fly) and the taxonomically close *D. neohumeralis*, both very serious pests of fruit and vegetable crops in eastern Australia. The same set of six relatively simple amides was found in each species, although in different proportions. Since that report, studies of a number of

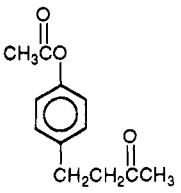
[†] University of Queensland.
[‡] Department of Primary Industries.
[§] Universität Tübingen.
[∥] Institut für Organische Chemie der Universität.

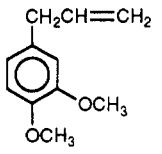
(1) (a) See, for example: Drew, R. A. I.; Hooper, G. H. S.; Baterman, M. A. *Economic Fruit-Flies of the South Pacific Region*; Workshop Text, Department of Primary Industries: Brisbane, 1978. (b) *Fruit-Flies of Economic Importance* Cavalloro, R., Ed.; Proceedings CEC/10BC Int. Symp. Athens, November, 1982; A.A. Balkema/Rotterdam, 1983.
(2) Fletcher, B. S. *J. Insect Physiol.* **1969**, *15*, 1309.
(3) Schultz, G. A.; Boush, G. M. *J. Econ. Entomol.* **1971**, *64*, 347.
(4) Haniotakis, G. E. *Environ. Entomol.* **1974**, *3*, 82. See also: Mazomemos, B. E.; Haniotakis, G. E. *J. Chem. Ecol.* **1981**, *7*, 437.
(5) Fletcher, B. S. *Nature* **1968**, *219*, 631.
(6) Ohinata, K.; Jacobson, M.; Kobayashi, R. M.; Chambers, D. L.; Fujimoto, M. S.; Higa, H. H. *J. Environ. Sci. Health* **1982**, *A17*, 197.
(7) Nation, J. L. *Environ. Entomol.* **1975**, *4*, 27.
(8) Battiste, M. A.; Strekowski, L.; Vanderbilt, D. P.; Visnick, M.; King, R. W.; Nation, J. L. *Tetrahedron Lett.* **1983**, *24*, 2611.
(9) Jacobsen, M.; Ohimata, K.; Chambers, D. L.; Jones, W. A.; Fujimoto, M. S. *J. Med. Chem.* **1973**, *16*, 248.
(10) Pritchard, G. *Aust. J. Entomol. Soc.* **1967**, *6*, 127.
(11) Prokopy, R. J. *Can. Entomol.* **1975**, *107*, 905.
(12) Katsoyannos, B. I. *Environ. Entomol.* **1976**, *4*, 801.
(13) Bellas, T. E.; Fletcher, B. S. *J. Chem. Ecol.* **1979**, *5*, 795.

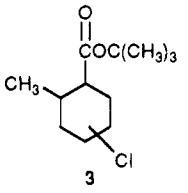
0022-3263/89/1954-3893$01.50/0

fruit-fly species have been conducted, and a range of chemically diverse structural types have been identified, representing amides, lactones, saturated and unsaturated alcohols and diols, esters, and spiroacetals. Fatty acids also occur.[14] 1,7-Dioxaspiro[5.5]undecane, from female olive flies (*D. oleae*), was the first spiroacetal to be identified as a specific insect sex pheromone.[15]

Australasia is rich in fruit-fly species[1] and regular quarantine monitoring and detection are conducted by using certain chemical lures in traps, e.g. **1** (Cue-Lure), **2** (methyleugenol), or **3** (Trimedlure), which are not pheromones.


1


2


3

The males of most tephritids in the South Pacific region are strongly attracted to **1** or **2**, whereas isomers of **3** are effective for the Mediterranean fruit fly.[1] In addition to the "Cue-Lure" group and the "methyleugenol" group, there is a third group of flies that does not respond to these lures and hence population variations and penetration of previously uninfested areas are much more difficult to detect.[1] Chief among these are *D. cucumis* (cucumber fly), *D. halfordiae*, *D. decipiens*, and *D. latifrons*. The cucumber fly is a major pest in Queensland (cucurbits, tomatoes, papayas) and is taxonomically close to *D. decipiens*, thought to be confined to New Britain, but potentially a major threat to Australian horticulture.[1] *D. halfordiae* is of restricted habitat in Queensland and of minor economic importance (citrus and loquat crops), but is taxonomically close to *D. cucumis*. *D. latifrons* is now located in Hawaii, as well as in S.E. Asia.

Our attention was directed to this latter group of flies (particularly *D. cucumis*) and our long term goal was the development of an attractant as well as providing data for taxonomic correlation, an area of considerable confusion and flux, particularly with *D. dorsalis* (oriental fruit fly)[16] and *Ceratitis capitata* (med-fly).[16] We have examined as part of a continuing program approximately 15 fruit-fly species from Australasia, and here we report in full our studies of the male cucumber fly, *D. cucumis* (French) and *D. halfordiae* (Tryon).[17]

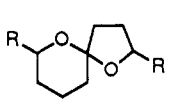
Results and Discussion

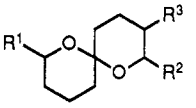
Dissection of male cucumber flies was conducted as described in the Experimental Section.[14] Extracts of dissected rectal glands were examined by capillary gas chromatography and it is seen that one major (~60%) and a number of minor components constitute the volatile portion of the glandular secretion. Consideration of the mass spectra confirmed that spiroacetals were predominant and three systems were represented: 1,6-dioxaspiro[4.5]-decanes **4**; 1,7-dioxaspiro[5.5]undecanes **5**; and 1,7-dioxaspiro[5.6]dodecanes **6**. A number of these occurred as diastereomeric mixtures.

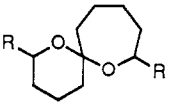
Table I. Volatile Components in the Solvent Extracts of the Rectal Gland of Adult Male Fruit Flies

compound[a]	relative amount (%)
Dacus cucumis (French)	
1. trimethylpyrazine	<1
2. tetramethylpyrazine	<1
3. (*EE*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane	~60
4. (*EE*)-2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane	~1
5. (*EZ*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane	5
6. 2-methyl-1,7-dioxaspiro[5.6]dodecane	~1
7. (*ZZ*)-2-ethyl-7-methyl-1,6-dioxaspiro[4,5]decane	~1
8. (*ZZ*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane	8
9. 2-(1′-hydroxyethyl)-7-methyl-1,6-dioxaspiro-[4.5]decane, isomer 1	<1
10. 2-(1′-hydroxyethyl)-7-methyl-1,6-dioxaspiro-[4.5]decane, isomer 2	<1
11. 3-hydroxy-2,8-dimethyl-1,7-dioxaspiro[5.5] undecane, isomer 1	~1
12. (*EE*)-2-(hydroxymethyl)-8-methyl-1,7-dioxaspiro[5.5]undecane	<1
13. 3-hydroxy-2,8-dimethyl-1,7-dioxaspiro[5.5] undecane, isomer 2	15
14. (*R*)-(−)-1,3-nonanediol	10
Dacus halfordiae (Tryon)	
1. (*E,E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane	~70
2. 2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane	~1
3. 2-methyl-6-pentyl-3,4-dihydro-2*H*-pyran	~1
4. diethyl succinate	12
5. (*E,E*)-2-ethyl-8-methyl-1,7-dioxaspiro[5.5] undecane	8
6. 6-oxo-1-nonanol	10

[a] Fatty acids were also present. For example, in *D. halfordiae* the following were identified: decanoic, dodecanoic, tetradecanoic, pentadecanoic, hexadecanoic (major).

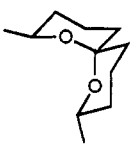

4

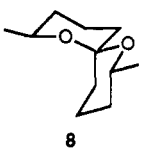

5


6

A full listing of the compounds observed and identified in the extracts of *D. cucumis* (along with approximate percentages) is shown in Table I and discussed below.

The major volatile component (~60%) exhibited a molecular ion at m/z = 184, a weak ion at 169 (M − CH_3), but intense ions at m/z 115 and 112 (100%). Consideration of the known fragmentation patterns of simple alkyl-substituted spiroacetals[18] indicated the compound to be a 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane,[19] and this was confirmed as the *E,E* diastereomer **7** by comparison of its mass spectrum and VPC characteristics with those of a synthesized authentic sample (see below).


7


8


9


10

Two later eluting components had very similar mass spectral patterns (M = 184) except that the m/z = 112 ion was now weaker than the m/z = 115, which was the base peak. VPC comparisons of the known and characterized

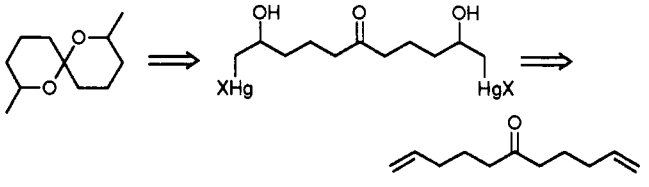
(14) A comprehensive discussion may be found in Lewis, J. A. Ph.D. Dissertation, University of Queensland, 1987.
(15) Baker, R.; Herbert, R.; Howse, P. E.; Jones, O. T.; Francke, W.; Reith, W. *J. Chem. Soc., Chem. Commun.* **1980**, 52.
(16) Private correspondence and discussions with Dr. R. A. I. Drew and Dr. B. S. Fletcher (CSIRO Canberra).
(17) Some of this work has been reported in preliminary form: Kitching, W.; Lewis, J. A.; Fletcher, M. T.; Drew, R. A. I.; Moore, C. J.; Francke, W. *J. Chem. Soc., Chem. Commun.* **1986**, 853.
(18) (a) Francke, W.; Reith, W. *Liebigs Ann. Chem.* **1979**, 1. (b) Francke, W.; Hindorf, G.; Reith, W. *Naturwissenschaften* **1979**, *66*, 619.
(19) Francke, W.; Reith, W.; Bergstrom, G.; Tengo, J. *Naturwissenschaften* **1980**, *67*, 149.

(^{1}H and ^{13}C NMR) *E,Z* diastereomer (which was acquired by a new procedure discussed below) confirmed the presence of the *E,Z* diastereomer **8**, and we concluded, somewhat tentatively at this stage, that the *Z,Z* diastereomer **9** of the same system was also present. **7** and **8** had been characterized previously by Francke,[20] and certain enantiomers of them were described by Mori.[21]

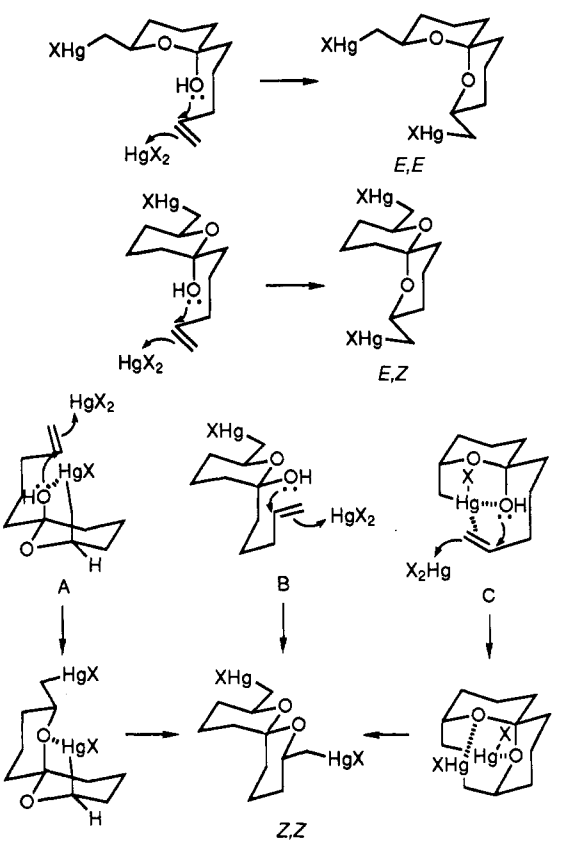
"Headspace volatile"[14] analyses were conducted by adsorption of the volatiles from a collection of 50 male *D. cucumis* (in a glass bell-jar chamber) onto special tubes loaded with Tenax GC to demonstrate that the major glandular components were indeed released into the atmosphere. Flash thermal desorption into the GC/MS system showed the headspace volatiles to consist predominantly of (*EE*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**7**) with very minor proportions of the *E,Z* diastereomer **8**, the presumed *Z,Z* diastereomer **9**, and the hydroxy spiroacetal **15**. Similar experiments with a collection of female flies showed a much lower level of these compounds in the atmosphere.

Our conclusion that the previously uncharacterized *Z,Z* diastereomer **9** was present at an appreciable level is of considerable interest, and characterization of a synthetic sample by NMR and GC-MS methods was considered very desirable. However, it was recognized that tactical acquisition of **9** would be difficult for the reasons outlined below. Reversible dehydrative spiroacetalization of the notional racemic keto diol 6-oxo-2,10-undecanediol in principle can provide three diastereomeric 2,8-dimethyl-1,7-dioxaspiro[5.5]undecanes, **7**, **8**, and **9**, with **7** and **9** together representing 50% of the total. The equilibrium between **7** and **9** would be heavily regulated by the anomeric effect[22] so that (*E,E*)-**7** would predominate to the virtual exclusion of **9** under mildly aqueous acidic conditions.[23] (Under these conditions, **7** or **9** would not equilibrate with **8**.) The estimated relative free energies of **7**, **8**, and **9** are 0, 2.4, and 4.8 kcal/mol, respectively.[22,23] Hence any approach to **9** should avoid equilibrating conditions and incorporate the possibility of significant stabilization of intermediates leading to **9**. This presumably must occur in vivo, where the **9**/**7** ratio is much greater than its equilibrium value. Metal ion coordination (e.g., calcium) to possible diol or ketol precursors may be providing an energetically feasible route to **9**, formed along with the predominating **7**.

Synthesis of *E,E*, *E,Z*, and *Z,Z* Diastereomers of 2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane. Simple retrosynthesis shows that (Markovnikov) oxymercuration of 1,10-undecadien-6-one should provide a keto diol, equivalent to the skeleton of the target system.[24] On the assumption that "double" oxymercuration would be stepwise, a number of hemiketal intermediates could be envisaged



Scheme I



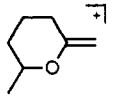
that would lead to *E,E*, *E,Z*, or *Z,Z* spiroacetal systems, when consummated by the second (intramolecular) oxymercuration step (Scheme I). Intermediates providing *E,E* and *E,Z* systems are unexceptional , whereas of the three (A, B, C in Scheme I) possible hemiacetals that could lead to the *Z,Z* spiro system, A and C are more attractive, as B enjoys no anomeric stabilization.[22] The unfavorable 1,3-diaxial interaction between methylene and oxygen (2.4 kcal/mol)[23] in A and C may be offset by the coordination of mercury to oxygen.[25] C has the added feature of mercury–ene coordination. Subsequent to oxymercuration of the second double bond, a single ring reversal for case A or two ring reversals for case C would lead to *Z,Z* spiroacetal with both CH_2HgX groups equatorial. Our hope was that the populations of A and/or C under irreversible conditions would be adequate to permit eventual isolation of some **9** for NMR and GC/MS studies.

1,10-Undecadien-6-one was prepared by addition of 4-pentenylmagnesium bromide to ethyl formate followed by Jones oxidation.[24] Oxymercuration was conducted with $Hg(OAc)_2$ in a 1:1 mixture of THF and H_2O (1% $HClO_4$), and the resulting mercuri-substituted spiroacetals could be isolated as acetates or chlorides, which were viscous oils or solids and relatively air and light stable. Essentially pure *EE* mercurial could be obtained by recrystallization[14,24] and >90% diastereomerically pure directly from the reaction when conducted under acidic conditions for extended periods (~15 h) to permit equilibration, as both spiroacetalization and oxymercuration are reversible under such conditions. However, by controlling the reaction conditions, significant quantities of the *E,Z* mercurial could be produced (~40%), particularly when $HClO_4$ was

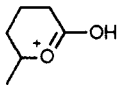
(20) Francke, W.; Reith, N.; Sinnwell, V. *Chem. Ber.* **1980**, *113*, 2686.
(21) (a) Mori, K.; Tanida, K. *Tetrahedron* **1981**, *37*, 3221. (b) Mori, K.; Watanabe, H. *Tetrahedron* **1986**, *42*, 295.
(22) For a full discussion, see: Delongschamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: Oxford, 1983.
(23) Delongschamps, P.; Rowan, D. D.; Pothier, N.; Sauve, T.; Saunders, J. K. *Can. J. Chem.* **1981**, *59*, 1106.
(24) For some preliminary discussion, see: Kitching, W.; Lewis, J. A.; Fletcher, M. T.; De Voss, J. J.; Drew, R. A. I.; Moore, C. J. *J. Chem. Soc., Chem. Commun.* **1986**, 855.
(25) In this connection, see: (a) Henbest, H. B.; Nicholls, B. *J. Chem. Soc.* **1959**, 227. (b) Bentham, S.; Chamberlain, P.; Whitham, G. H. *Chem. Commun.* **1970**, 1528. (c) Giese, B.; Bartmann, D. *Tetrahedron Lett.* **1985**, *26*, 1197.

omitted and reaction times were minimized (<10 min). Under these conditions a low level of a third diastereomer (presumed to be the *Z,Z*) was observed on the basis of ^{13}C signals in the spiro-carbon region for these 2,8-bis(chloromercurimethyl)-1,7-dioxaspiro[5.5]undecanes: *E,E* 97.36 ppm, *E,Z* 98.30 ppm, and *Z,Z* 101.90 ppm (benzene–pyridine-d_5 solvent).

The *E,E* and *E,Z* mercurials were fully characterized by ^1H and ^{13}C NMR and full details are located elsewhere.[14] Reductive demercuration of this latter mixture provided **7** (48%), **8** (40%), and the slowest eluting isomer (capillary GC; OV101) (3%) concluded below to be **9**. The EIMS of **7**, **8**, and (presumed) **9** and of their 5,5,11,11-tetradeuterio isomers are very similar and establish the 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane constitution.[18] The major difference concerns the relative intensities of the ion **11** (*m/z* 112; 116 in tetradeuterio analogue), which is the base peak in **7** whereas *m/z* = 115 (**12**) is the base peak in **8** and **9**. (These differences are apparent also in the mass spectra of the natural components.)



11



12

Very careful preparative gas chromatography of this mixture resulted in the isolation of the known **7** and **8** (each >90% diastereomerically pure), and presumed **9** (~85%). As required by their symmetry, **7** and **8** exhibit six (δ (C_6D_6) ppm: 19.42, 22.34, 33.31, 35.72, 65.23, 96.11) and eleven (19.11, 19.23, 22.12, 22.34, 30.35, 32.60, 33.53, 36.61, 65.93, 68.54, 97.12) ^{13}C NMR lines, respectively,[20] and in the ^1H NMR spectra (C_6D_6) one (δ 3.73) and two absorptions (δ 3.42; 4.26) respectively for >CH–O and also for the methyl groups (**7**, δ 1.15; **8**, δ 1.14, 1.19). That the stereoisomer concluded to be **9** possessed a 2-fold axis was confirmed by its six-line ^{13}C NMR spectrum (δ (C_6D_6) ppm: 19.46, 22.86, 30.88, 32.39, 68.50, 98.78) and the 400-MHz ^1H spectrum (C_6D_6) with one low-field absorption (δ 3.60 with J = 9.3, 6.4, and 3.4 Hz; >CH–O) and a single methyl doublet (δ 1.22; J = 6.4 Hz). The large coupling constant (9.3 Hz) (J_{2-3ax}) for H2 (δ 3.60) in **9** indicated this proton to be predominantly axial (*equatorial* CH_3) so that the conformation depicted for **9** is the dominant one, with no anomeric stabilization. However some distortion of the chair geometry may operate. Full anomeric gain could be achieved by ring-reversals, placing both methyl groups *axial*, but the nonbonded interactions are then severe. The 400-MHz ^1H NMR spectrum of **9** is reproduced in Figure 1, and the resonances of >C*H*–O (when compared with those for the *E,E* and E,*Z* isomers) indicates such protons experience a 1,3-diaxial relationship with carbon, not oxygen, following, for example, the analysis of Mori[21b] on similar systems. In addition, no other ring protons in the *Z,Z* diastereomer **9** appear below δ 1.65, strongly indicative of the absence of any axially disposed C–O bonds. (In **7** and **8**, ring proton resonances (other than >C*H*–O) appear as low as δ 2.05 and 1.93, respectively.) More detailed discussion of the ^1H and ^{13}C NMR spectra of **7**, **8**, and **9** may be found elsewhere.[14]

Equilibration studies provided further evidence for the constitution of **9**. Exposure of **9** (contaminated with ca. 6% of each of **7** and **8**) to aqueous acid resulted in facile equilibration (<5 min) to a mixture of **7** (94%) and **8** (6%) with **9** now undetectable by capillary VPC. This is required[22,23] if **9** is the *Z,Z* diastereomer, as it can isomerize to the *E,E* diastereomer **7** only if equatorial methyl groups are maintained and configurational changes at the notional

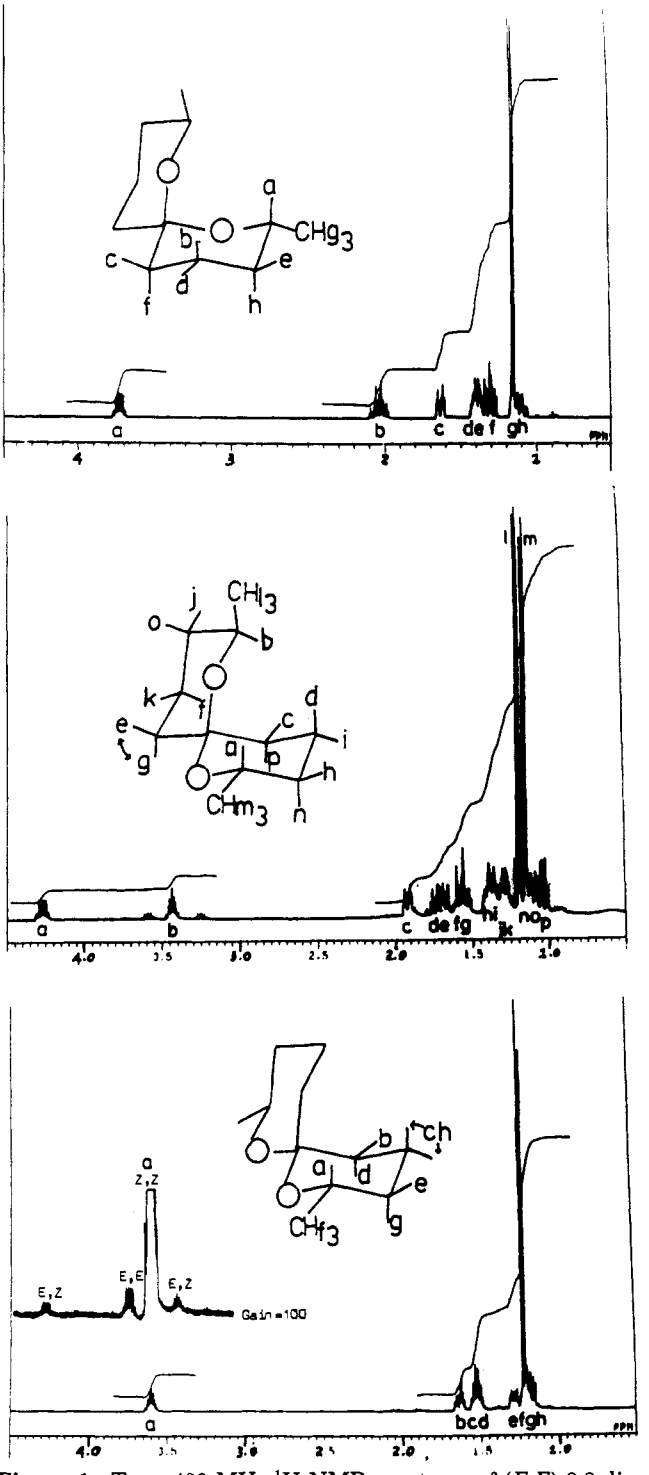


Figure 1. Top: 400-MHz ^1H NMR spectrum of (*E,E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (C_6D_6 solvent), showing the anticipated one methyl doublet and one >C*H*–O absorption. Middle: spectrum of (*E,Z*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, showing a duality of methyl doublets and >C*H*–O absorptions. Bottom: spectrum of (*Z,Z*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, showing one methyl doublet and one >C*H*–O absorption. (In the latter region, residual signals for the *E,E* and *E,Z* diastereomers are indicated.) See text for discussion.

secondary alcohol centers do not occur under such mildly acidic conditions.

With **7**, **8**, and **9** available, combined GC-MS examinations confirmed that these diastereomers were present in the cucumber fly secretion to the extent of 60%, 5%, and 8%, respectively. Considering the relative energies of **7** and **9**, this result is intriguing, and the possible role of

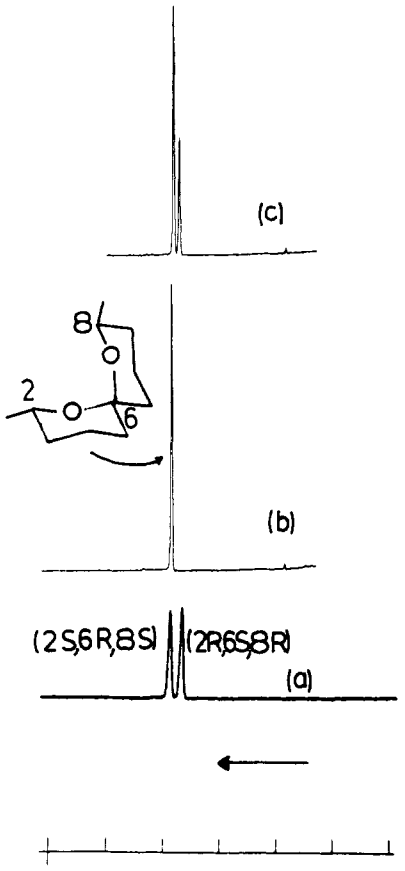
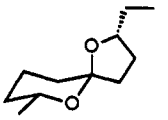


Figure 2. (a) Enantiomer resolution of (*E,E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane. Conditions: 40 m glass capillary column with per-*n*-hexyl-α-cyclodextrin. Column temperature 80 °C; carrier gas 1 bar H_2. Approximate retention time is 10 min. (b) Corresponding trace for the glandular extract of *D. cucumis*, showing the presence of the 2*S*,6*R*,8*S* enantiomer only. (c) Trace of natural sample (as in b) mixed with (±)-(*E,E*) diastereomer as in a.

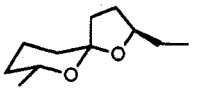
metal ions in vivo has been alluded to above. It is conceivable that the presence of thermodynamically less favored isomers would furnish an "inbuilt clock" which would provide information on the age of the bio-signal, given that the **9**/**7** ratio observed is well removed from the equilibrium value.

Chirality of 7, 8, and 9. The importance of chirality on the level of response by fruit flies to a given compound was demonstrated[26] by the attraction of male and female olive flies (*D. oleae*) to the enantiomers of 1,7-dioxaspiro[5.5]undecane (**10**). Consequently attempts were made to determine the enantiomeric composition of **7**, **8**, and **9**, employing complexation gas chromatography with metal-bearing chiral ligands as developed by Schurig and coworkers.[27] The *E,Z* diastereomer **8**, as secreted by male flies, was nicely resolved into enantiomers on chromatography and was racemic. However, this and similar chiral phases[27] did not resolve the enantiomers of the most abundant *E,E* diastereomer **7** and this is consistent with the view that complexation through oxygen to the metal center appears least favorable for this diastereomer. Conclusions regarding the nature of the least stable **9** were not clear cut, under the above chromatographic conditions. Fortunately, enantiomeric separation of spiroacetal systems, using hydrophobic derivatives of cyclodextrins as chiral phases, has recently been achieved.[28] In particular, the enantiomers of (*E,E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**7**) are nicely resolved. As shown in Figure 2, appropriate capillary gas chromatographic determinations, using per-*n*-hexyl-α-cyclodextrin as stationary phase, establish that the natural *E,E* diastereomer is highly enantiomerically pure, possessing the 2*S*,6*R*,8*S* configuration[21] as shown above in **7**. 1,7-Dioxaspiro[5.5]undecane (**10**), as secreted by *D. cacuminatus* and *D. oleae* (olive fly), has been shown to be racemic,[17,26] but here the chirality is associated with the sometimes labile spiro center, whereas (formal) secondary alcohol centers are present in **7**, **8**, and **9**. **8** has opposite chirality at the secondary centers (C_2, C_8) and if **8** results from ring closure of a "preformed" keto diol, the hydroxylation steps must have provided (some) secondary alcohol centers of opposite chirality, but the extent of this regulates the amount of **8** that is possible. (The enantiomers of **7** and **9** have like chirality at C_2 and C_8.) It follows that some keto diol with 2*R*,10*R* stereochemistry would be formed also, but the resulting 2*R*,6*S*,8*R* enantiomer of the *E,E* diastereomer would be present at a very low level.

Minor dialkyl spiroacetals in the *D. cucumis* secretion included (*E,E*)- and (*Z,Z*)-2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane (**13** and **14**), which were identified by comparison of GC-MS characteristics with those of authentic samples. Previously the *E,E* and *E,Z* diastereomers had



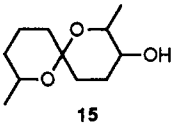
13



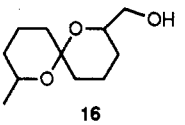
14

been identified in other insects,[19] but there have been no reports of the *Z,Z* diastereomer. 2-Methyl-1,7-dioxaspiro[5.6]dodecane was also identified (GC/MS comparison with a published spectrum)[24] as a minor component.

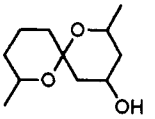
Hydroxy-substituted spiroacetals were also considered to be present and one in significant amount (15%). Compared with **7**, the mass spectrum exhibited ions indicating the presence of an additional oxygen atom, e.g., m/z = 156, 128, and an apparent M = 200. A likely candidate was 3-hydroxy-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**15**) (on the basis that dehydration and retro-Diels–Alder breakdown would provide the base peak at m/z = 112) but isomers **16**, **17**, and even **18** were considered and synthesized for comparison.



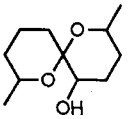
15



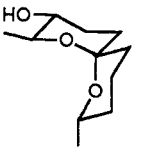
16



17



18

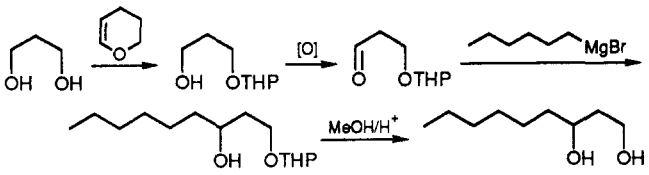


19

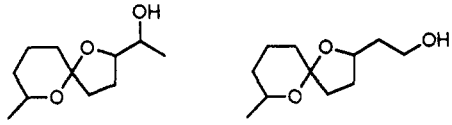
GC-MS comparisons with a synthetic sample of **15** (formed with another isomer (**20**)) confirmed the presence of **15**. This was subsequently shown to be *E,E,E* diastereomer **19** based on VPC comparisons and 2D-NMR analyses of separately synthesized diastereomers of **15**, uncontaminated with **20**. A second isomer of **15** was present in a very minor amount. (The synthesis of com-

(26) Haniotakis, G.; Francke, W.; Mori, K.; Redlich, H.; Schurig, V. *J. Chem. Ecol.* **1986**, *12*, 11.
(27) Weber, R.; Schurig, V. *Naturwissenschaften* **1984**, *71*, 408.
(28) (a) König, W. A.; Lutz, S.; Wenz, G. in press. (b) Private correspondence with Professors König, Francke (Hamburg), and Schurig (Tübingen).

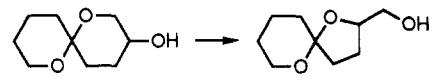
Scheme II



pounds **15**–**18** and other hydroxy spiroacetals will be reported in detail subsequently.[30]) **15** is a minor component of *D. tryoni* (Froggatt) and is the first spiroacetal observed in a male dacine fly attracted to Cue-lure. The three remaining hydroxy spiroacetals were present as very minor components (<1%). The first was (*E*,*E*)-(**16**),[24,31a] which was independently synthesized and showed M = 200 with m/z = 169 (M – CH_2OH), 131, and 128, indicative of hydroxylation, whereas the remaining two were considered to be isomers of either **20** or **21**. Synthesis[30] of isomers



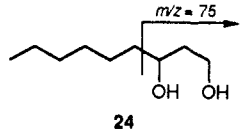
20 **21**



22 **23**

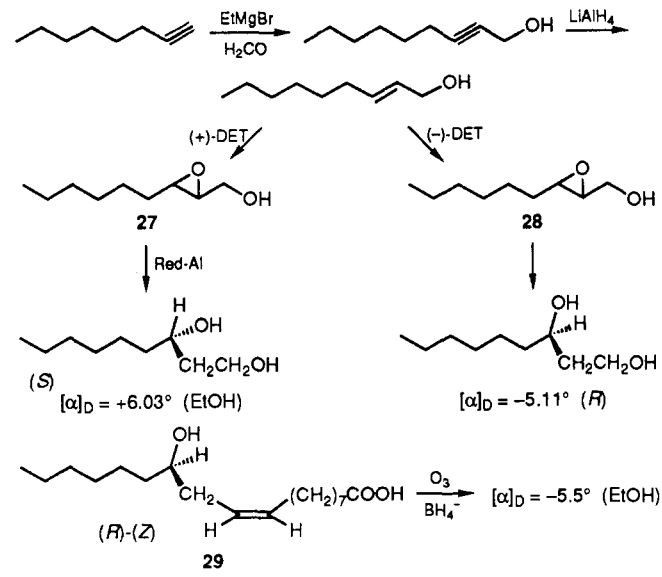
of **20** confirmed their presence in the fly secretion. Baker reported[32] the thermal conversion (GC/MS conditions) of **22** to **23** and similar behavior of **15** could provide **20**, and thus it is uncertain whether **20** is a natural compound. A number of hydroxy-substituted spiroacetals, e.g., **16**, **17**, have been identified in the mandibular gland secretions of species of Andrena bees.[31] Mori has suggested on the basis of private correspondence with Bellas that 1,7-dioxaspiro[5.5]undecane (**10**) is a minor component of the *D. cucumis* secretion.[33] However, examination of many acetone or pentane extracts of this fly from varied sources has failed to reveal this spiroacetal. It is possible that the original specimens of *D. cucumis* were contaminated with a *D. cacuminatus* specimen whose very predominant glandular component (ca. 8 μg/fly) is this simple spiroacetal.[17]

Other Compounds. High molecular weight hydrocarbons and fatty acids were observed together with trimethyl- and tetramethylpyrazine (<1% in some extracts). A further component (~11% of the volatiles) with highest m/z = 113 (30%) and 75 (100%) (EIMS) was also present, and chemical ionization techniques identified ions at 159 (M – 1) and 142 (– H_2O) providing M = 160.[34] This component was suspected to be 1,3-nonanediol (**24**).



24

Scheme III

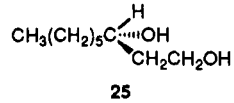


29

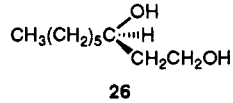
1,3-Nonanediol was initially synthesized by the method in Scheme II. 1,3-Propanediol was selectively protected with dihydropyran as described by Kozluk[35] and separated from other products by silica gel chromatography. (A product not reported by Kozluk was considered to be the 1,3-dioxane derivative of 5-hydroxypentanal, formed by rearrangement of the monoprotected 1,3-propanediol.) Oxidation with pyridinium chlorochromate buffered with sodium acetate provided the protected hydroxy aldehyde, which on reaction with *n*-hexylmagnesium bromide and deprotection afforded 1,3-nonanediol, identical in all respects (GC, EIMS and CIMS) with the natural material. The base peak (m/z = 75) was provided by the fragmentation shown on **24**.

In view of the demonstrated importance of chirality in some semiochemicals[36] and the possible biosynthetic connection between diols and spiroacetals, determination of the chirality of this natural diol was considered worthwhile. This involved enantioselective syntheses of both (*S*)-(+) and (*R*)-(–)-1,3-nonanediols using Sharpless asymmetric epoxidation[37] as a key step and chiral gas chromatographic analyses of these and of the natural diol.[38]

Reaction of octynylmagnesium bromide with gaseous formaldehyde provided 2-nonyn-1-ol (86%), which on reduction ($LiAlH_4$) yielded exclusively (*E*)-2-nonen-1-ol (76%) together with a minor amount of 1,2-nonadiene, which was easily separated. Epoxidation (MCPBA) to 2,3-epoxy-1-nonanol and reduction with Red-Al (THF; –30 °C → –10 °C) provided exclusively 1,3-nonandiol (80%). Hence Sharpless asymmetric epoxidation[37] of (*E*)-2-nonen-1-ol followed by Red-Al reduction[39] would represent enantioselective routes to (+)- and (–)-1,3-nonanediol (**25** and **26**). (See Scheme III.)



25



26

(29) Francke, W.; Reith, W.; Bergström, G.; Tengö, J. *Z. Naturforsch.* **1981**, *36C*, 928.
(30) Manuscript in preparation.
(31) (a) Bergström, G.; Tengo, J.; Reith, W.; Francke, W. *Z. Naturforsch* **1982**, *37C*, 1124. (b) Francke, W. *Nova Acta Regiae Soc. Sci. Upsaliensis* **1984**, Serie V:C,3,119.
(32) Baker, R.; Herbert, R. H. *J. Chem. Soc., Perkin Trans. I* **1987**, 1123.
(33) Mori, K.; Uematsu, T.; Kazunori, Y.; Minobe, M. *Tetrahedron* **1985**, *41*, 2751.
(34) We are grateful to Dr. J. McLeod, Research School of Chemistry, Australian National University (Canberra) for these measurements.
(35) Kozluk, T.; Cottier, L.; Descotes, G. *Tetrahedron* **1981**, *37*, 1875.
(36) Silverstein, R. M. in *Semiochemistry, Flavors and Pheromones*; Proceedings of the American Chemical Society Symposium, Washington, 1983. Mori, K. In *Techniques in Phermone Research*; Hummel, H. E., Miller, T. A., Eds.; Springer-Verlag: New York, 1984, p 323.
(37) Sharpless, K. B.; Woodard, S. S.; Finn, M. G. *Pure Appl. Chem.* **1983**, *55*, 1823.
(38) König, W. A.; Steinbach, E.; Ernst, K. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 527.
(39) Finan, J. M.; Kishi, Y. *Tetrahedron Lett.* **1982**, *23*, 2719.

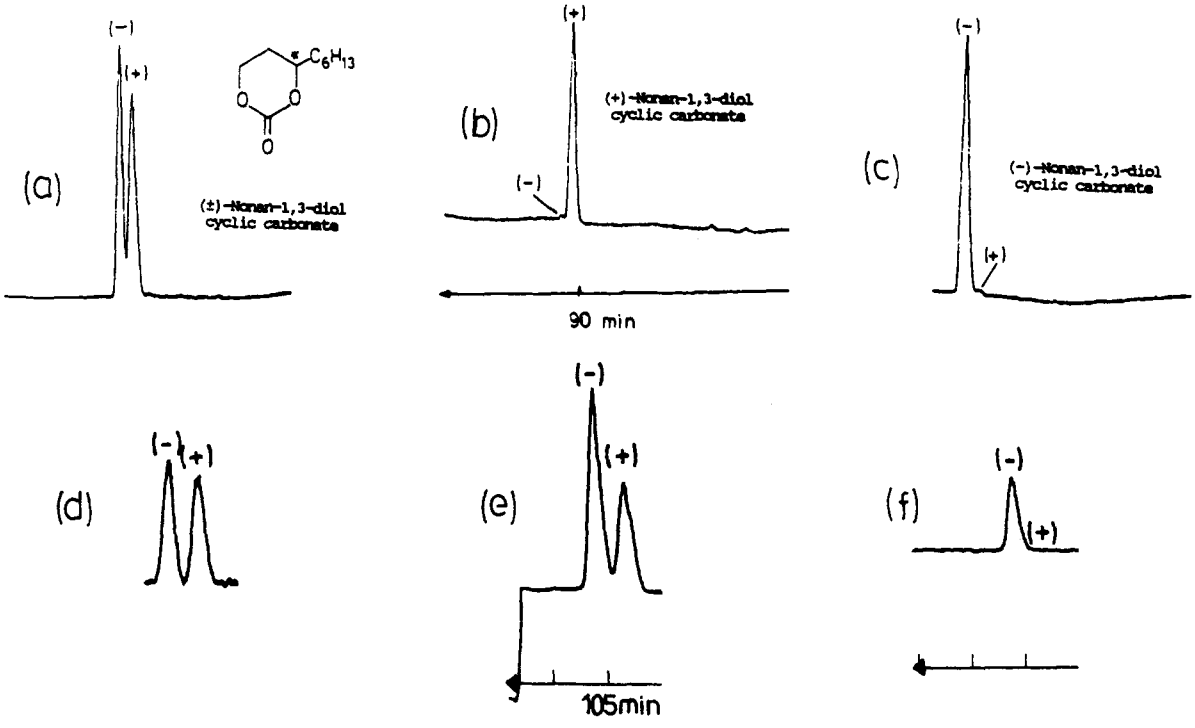
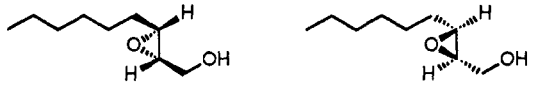
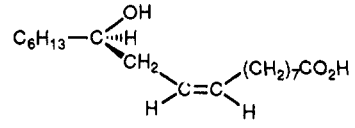


Figure 3. (a–c) Enantiomer separation of the standard samples of (a) (±)-nonane-1,3-diol, (b) predominantly (+), and (c) predominantly (–) enantiomers, as synthesized. Conditions: 50 m fused silica capillary column with XE-60–L-valine–(*R*)-α-phenylethylamide. Column temperature: 100–180 °C, temperature programmed 2 °C/min. Carrier gas: 1 bar H_2. (d–f) Comparison of natural sample: (d) (±)-nonane-1,3-diol, (e) natural sample mixed with (±)-nonane-1,3-diol, and (f) natural sample. Conditions: same column. Column temperature 170 °C. Carrier gas 1 bar H_2. Retention time 105 min.

Thus asymmetric epoxidation in the reported way[37] of (*E*)-2-nonen-1-ol[40] with L-(+)-diethyl tartrate provided (–)-2,3-epoxy-1-nonanol (**27**) (mp 45–45.5 °C; $[\alpha]^{34}_D$ = –38.9°; *c* = 0.0175 in $CHCl_3$), whereas D-(–)-diethyl tartrate yielded the (+) enantiomer ($[\alpha]^{20}_D$ = +38.7°; *c* = 0.0295 in $CHCl_3$) **28**. Precedent would indicate that the (+) enantiomer was the 2*R*,3*R* epoxy alcohol and both enantiomers were obtained in high optical purity on the basis of chiral gas chromatography (Figure 3) of their cyclic carbonates (diol + phosgene).[38] That the (+) epoxy alcohol had the 2*R*,3*R* configuration as shown in **28** was confirmed



27 **28**



29

in the following way. Ozonolysis of (*R*,*Z*)-(+)-12-hydroxy-9-octadecenoic acid (**29**) (riconoleic acid) of established absolute configuration,[41] followed by reduction ($NaBH_4$) of the resulting (*R*)-3-hydroxynonanal, provided (*R*)-(–)-1,3-nonandiol ($[\alpha]^{22}_D$ = –5.5°, *c* = 5.8, ethanol) as compared with –5.11° (*c* = 1.04, ethanol) and +6.03° (*c* = 1.2, ethanol) for the (*R*)-(–)- and (*S*)-(+)-1,3-nonandiols obtained by Red-Al reduction of the synthesized 2,3-epoxy-1-nonanols.

Treatment of the glandular secretion extract of male cucumber flies with phosgene (to form the cyclic carbonate of the diol) followed by chiral gas chromatographic analysis (Figure 3) using the carbonates of the (*R*)-(–)- and (*S*)-(+)-1,3-nonanediols as standards demonstrated the presence of the *R*-(–) enantiomer only. This information will permit more soundly based evaluation of the behavior of cucumber flies to the diols, and it is of interest that secondary hydroxylation to form **26** and the (notional) keto diol leading to **7** proceed with the same chiral sense. (The opposite *R* and *S* descriptors result from priority changes.)

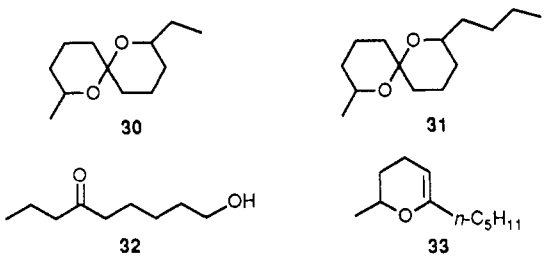
Glandular Secretion of *Dacus halfordiae* (Tryon). The major volatile component (~70%) of male rectal glands was (*EE*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**7**), as observed for the cucumber fly, but of considerable interest was the absence of the *E*,*Z* (**8**) and *Z*,*Z* (**9**) diastereomers. The absence of **8** suggests enantiospecific hydroxylation (in the same chiral sense) in the formation of the notional keto diol intermediate. However, chirality determinations with **7** have not yet been conducted. A minor component (~6%) with M = 198 and *m*/*z* = 169 (M – C_2H_5) and characteristic spiroacetal fragmentation (compared with 7-ethyl-2-methyl-1,7-dioxaspiro[4.5]decane) was considered to be the very unusual even-carbon numbered 2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane (**30**) apparently occurring as the *E*,*E* diastereomer.[17] This was confirmed by GC-MS comparisons with an authentic sample, originally synthesized in our laboratories by Fletcher.[17] This unusual spiroacetal is also found in specimens of *D. dorsalis* (oriental fruit fly),[17,42] *D. latifrons* (Hendel),[17] and *D. occipitalis* (Bezzi).[17] (Another even-carbon numbered spiroacetal, 2-butyl-8-methyl-1,7-dioxaspiro[5.5]undecane (**31**), is a minor component in *D. latifrons*.[17])

A further significant volatile component (~10%) was demonstrated to be 6-oxo-1-nonanol (**32**) by comparisons with a synthesized sample.[17] This keto alcohol also occurs

(40) For the asymmetric epoxidation of (*E*)-nona-2,8-dienol, see: Bulman-Page, P. C.; Rayner, C. M.; Sutherland, I. O. *J. Chem. Soc., Chem. Commun.* **1986**, 1408.

(41) Serck-Hanssen, K. *Chem. Ind.* **1958**, 1554.

(42) Baker, R.; Bacon, A. J. *Experientia* **1985**, *41*, 1484.



as the major component (65%; 5 μg/fly) in *D. occipitalis*.[17] The demonstration that **30** and **32** are found in both *D. occipitalis* and *D. halfordiae* is of interest, considering that males of the former species are strongly attracted to methyleugenol whereas the latter are not. We are unaware of any previous demonstration of 6-oxo-1-nonanol as an insect component other than in *D. occipitalis*.

A minor component of the gland extract displayed an apparent M = 168 ($C_{11}H_{20}O$) with prominent m/z = 125 and 112 (100%) ($C_7H_{12}O$), indicative of a dihydropyran nucleus. This was shown to be 2-methyl-6-n-pentyl-3,4-dihydro-2*H*-pyran (**33**) by comparison of its mass spectrum with that of an authentic sample.[31b] One side-chain oxygenation of **33** could then lead to the 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane system. **33** is a major component of the male cephalic secretion of *Andrena wilkella* species.[31a] We reported previously[17] that **8** (M = 184) was present in the *D. halfordiae* secretion, but higher quality mass spectra of this minor component revealed it to be 2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane (**13** or **14**).

Since the identification of 2-ethyl-1,6-dioxaspiro[4.4]-nonane as the principal aggregation pheromone of the bark beetle (*Pityogenes chalcographus*) by Francke in 1977,[43] spiroacetals have been identified in three different orders of insects and elicit a broad range of behavior responses. With a few exceptions (some described herein) spiroacetal pheromones (in orders Coleoptera, Hymenoptera, and Diptera) show unbranched carbon skeletons with nine, eleven, or thirteen carbon atoms[44] and hence are closely related to other straight-chain pheromones that are presumably fatty acid derived. Possible biogenetic connections indicate that spiroacetals may be found, for example, in Lepidopteran species where functionalized straight-chain systems abound. As pointed out elsewhere,[44] the possible male sex pheromone of a pyralide moth, (*Z*)-2,6-nonadien-4-olide,[45] possesses the functionality needed for smooth, biogenetic conversion to a spiroacetal. In the wild olive fly, the co-occurrence of 1,7-dioxaspiro[5.5]undecane and ethyl 6-oxoundecanedioate has been reported[46] and the connection here is clear.

Our (and other)[32,42] examinations show that a large variety of spiroacetal types are present in rectal gland secretion of a wide range of Dacus species. In most cases, the exact role and function of these compounds have not been determined, but biological evaluation and field assessments of some of those compounds are currently underway and may lead to specific attractants and controls for some Dacine species.

Experimental Section

Fruit Fly Glandular Extracts. Dissections were carried out on sexually mature male flies that had been laboratory reared on a standard artificial diet and fed as adults on protein hydrolysate, sugar, and water. Dissection of the male flies was performed by gently squeezing (tweezers) the abdomen in the region of the first and second segments, thus forcing the ninth segment containing the genitals to protrude slightly. The whole ninth segment was removed and this included the secretory sac and reservoir. The excised glands were then extracted in a suitable solvent and normally both pentane and acetone were employed.[14]

GC/MS spectra were recorded on a Hewlett-Packard Model 5992B instrument fitted with OV1 or BP5 capillary columns, whereas gas chromatographic analyses were performed on a Hewlett-Packard 5710A gas chromatograph using OV1 or BP5 capillary columns or a Varian Model 3700 gas chromatograph using an OV101 capillary column. Preparative gas chromatography was performed on a Shimadzu gas chromatograph Model GC-9A equipped with OV101 and C-20M columns. Headspace analyses and some GC-MS runs were performed on a Finnigan Mat 1020 automated GC/MS equipped with either the specially designed desorption unit or a Perkin-Elmer ATD50 automatic thermal desorption system. Mass spectra were recorded on an AEI-MS902 spectrometer at an ionizing voltage of 70 eV. IR spectra were recorded on a Perkin-Elmer Model 397 spectrometer. Optical rotations were measured on a Perkin-Elmer 141 MC polarimeter. ^{1}H NMR spectra were recorded at either 100 MHz (CW mode) on a JEOL JNM-PS100 spectrometer or at 100, 300, or 400 MHz in the FT mode on JEOL JNM-FX100, Bruker CXP-300, and JEOL JNM-GX400 spectrometers, respectively. Chemical shifts were references to internal tetramethylsilane (0.0 ppm) or residual $CHCl_3$ (7.24 ppm). ^{13}C NMR were recorded at 25.05 MHz, 75.46 MHz, or 100 MHz and chemical shifts were referenced to the center peak of the solvent ($CDCl_3$) at 77.00 ppm or benzene-d_6 (128 ppm).

Synthesis of (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane (7, 8, and 9). Undeca-1,10-dien-6-one. To the cooled (0 °C) Grignard reagent prepared from 5-bromo-1-pentene (10 g, 67 mmol) and magnesium (1.8 g, 74 mmol) in ether (60 mL) was added ethyl formate (2.6 g, 34 mmol). The reaction was warmed to room temperature and stirred for 1 h. The reaction was quenched with saturated ammonium chloride and extracted with ether. The separated ether fraction was dried and evaporated to yield a yellow oil, consisting of 1,10-undecadien-6-ol (69%) and the corresponding formate ester (31%). The crude product was refluxed in 15% aqueous KOH solution for 3 h to hydrolyze the ester, after which the cooled solution was extracted with ether. Solvent removal, etc., followed by distillation (112 °C/10 mm) provided the alcohol in 70% yield. ^{1}H NMR: 6.2 (2 H, m, CH=), 5.5 (4 H, m, CH_2=), 4.1 (1 H, br s, C*H*OH), 2.6 (4 H, m, allylic H), 2.0 (9 H, m, including OH). ^{13}C NMR: 138.71, 114.87, 71.58, 36.89, 33.73, 24.92. MS (m/z, rel intensity) (84, 20.2), (81, 98.7), (79, 22.9), (67, 25.1), (57, 39.8), (55, 98.4), (54, 38.0), (43, 60.6), (41, 100), (39, 53.7). Oxidation of the alcohol using Jones reagent was conducted in the standard way to provide the title ketone (100 °C/16 mm; 92%). ^{1}H NMR: 6.4 (2 H, m, CH=), 5.7 (4 H, m, CH_2=), 23–3.3 (12 H, m). ^{13}C NMR: 210.91, 137.99, 115.17, 41.96, 33.12, 22.85. MS: (112, 19.0), (97, 33.4), (84, 24.1), (70, 11.7), (69, 44.7), (58, 38.4), (55, 43.2), (43, 25.8), (41, 100).

Oxymercuration. The conditions under which the oxymercuration/reduction sequence was performed greatly affected the diastereomeric ratio in the product spiroacetals.[14,24,30] The procedure developed to optimize the proportions of the thermodynamically less favored diastereomer is outlined. To a stirred solution of undeca-1,10-dien-6-one (0.5 g, 3.0 mmol) in THF–H_2O (15 mL:15 mL) was added $Hg(OAc)_2$ (1.94 g, 6.1 mmol) in one portion and the homogeneous solution was stirred for 15 min. Benzyltriethylammonium chloride (2.4 g, 10.5 mmol) in 10% aqueous sodium hydroxide (15 mL) and dichloromethane (5 mL) was added followed by $NaBH_4$ (0.09 g, 2.3 mmol) in 10% aqueous sodium hydroxide (5 mL). The grey inhomogeneous reaction mixture was stirred for 15 min or until the solution was clear with a small pool of metallic mercury. The solution was filtered through Celite, which was washed with ether. The aqueous phase was extracted with ether and the combined organic phase was dried ($MgSO_4$) and evaporated and the product purified by Kugelrohr distillation (bp 90 °C; 15 mm (lit.[20] bp 86–87 °C; 17 mm)). Under these conditions, a mixture of *EE* (48%), *EZ* (40%), and *Z*,*Z* (3%) diastereomers of 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane formed, together with some dienone (~1%) and two isomers (~3%) of

(43) Francke, W.; Heemann, V.; Gerken, B.; Renwick, J. A. A.; Vité, J. P. *Naturwissenschaften* **1977**, *64*, 590.
(44) Francke, W. In *Les Mediateurs chimiques*; INRA: Versailles, 1982; p 81.
(45) Kuwahara, Y. *Appl. Ent. Zool.* **1980**, *15*, 478.
(46) Mazomenos, B. E.; Pomonis, J. G. in ref 1b, p 96.

2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane. Increasing the reaction time for oxymercuration to ca. 15 h and use of 1% aqueous $HClO_4$–THF as solvent led to very predominantly *E,E* diastereomer (>90%) with some *E,Z* (~6%) and no *Z,Z* isomer. (The intermediate organomercurials were easily isolated and characterized, and these compounds have been discussed elsewhere.[14])

The mixture of (*E,E*)-, (*E,Z*)-, and (*Z,Z*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecanes (**7**, **8**, and **9**) was separated by careful preparative gas chromatography. The *E,E* and *E,Z* diastereomers were each obtained >90% isomerically pure and the *Z,Z* ca. 85% pure. These were characterized by ^1H, ^{13}C, and 2-D NMR and GC/MS, with the salient features of the NMR data being discussed in the text.

(*E,E*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane (7). ^{13}C NOE δ (C_6D_6): 96.11 (s), 65.23 (d, J_{C-H} = 141 Hz), 35.7 (t, J = 125 Hz), 33.31 (t, J = 123 Hz), 22.34 (q, J = 125 Hz), 19.42 (t, J = 128 Hz). MS (*m/z*, rel intensity): (184, 15.0, M^+), (140, 15.0), (115, 96.1), (114, 36.7), (112, 100), (97, 60.3), (69, 39.4), (55, 55.0), (43, 70.1), (41, 64.8). Anal. Calcd for $C_{11}H_{20}O_2$: C, 71.7; H, 10.9. Found: C, 71.3; H, 11.2.

(*E,Z*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane (8). ^{13}C NOE δ (C_6D_6): included 97.3 (s), 68.5 (d, J = 140 Hz), 65.9 (d, J = 140 Hz), 22.4 (q, J = 120 Hz), 22.1 (q, J = 120 Hz). (See text for full listing). MS (*m/z*, rel intensity): (184, 10.2, M^+), (115, 100), (114, 33.5), (112, 42.6), (97, 62.4), (69, 45.9), (55, 44.5), (43, 48.6), (42, 33.6), (41, 50.4).

(*Z,Z*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane (9). MS (*m/z*, rel intensity): (184, 11.3, M^+), (115, 100.0), (114, 36.2), (112, 49.4), (97, 66.9), (69, 44.0), (55, 45.9), (43, 45.9), (42, 34.2), (41, 50.2).

Oxymercuration–reduction of 5,5,7,7-tetradeuterio-1,10-undecadien-6-one (obtained by base-catalyzed H–D exchange) led to the 5,5,11,11-tetradeuterio derivatives of the above diastereomers, such deuteration assisting in the analysis of the NMR spectra (see text). MS of tetradeuterioanalogues: (*E,E*) (188, 16.1, M^+), (144, 18.6), (117, 87.0), (116, 100.0), (115, 63.1), (99, 31.3), (71, 25.2), (45, 34.0), (43, 58.1), (42, 37.6); (*E,Z*) (188, 9.6, M^+), (117, 100.0), (116, 64.5), (115, 53.6), (114, 13.2), (99, 44.6), (71, 33.4), (55, 26.9), (43, 55.8), (42, 41.0); (*Z,Z*) (188, 0, M^+), (117, 84.9), (116, 71.7), (115, 56.6), (114, 20.8), (99, 43.3), (71, 45.3), (55, 50.9), (43, 100.0), (42, 73.6), (41, 50.9).

Synthesis of (±)-1,3-Nonanediol (24): Procedure A. 3-(Tetrahydropyranyloxy)propan-1-ol was prepared from propane-1,3-diol as reported[35] and purified by silica gel chromatography. 1,3-Bis(tetrahydropyranyloxy)propane was eluted with hexane and 3-(tetrahydropyranyloxy)propan-1-ol with hexane/ether (2:1). An additional product, considered to be the 1,3-dioxane derivative of 5-hydroxypentanal, was also isolated. ^{13}C NMR: 99.0, 65.80, 62.40, 60.88, 32.04, 30.55, 25.23, 19.55. (Dioxane derivative: 102.19, 66.88 (2 C), 62.67, 34.70, 32.42, 25.81, 20.11.) **3-(Tetrahydropyranyloxy)propanal** was obtained (93%) by pyridinium chlorochromate oxidation of the above alcohol under NaOAc-buffered conditions in dichloromethane, in the usual way. ^{13}C NMR: 201.24, 98.95, 62.21, 61.18, 43.82, 30.46, 25.33, 19.30. The unpurified aldehyde (0.9 g, 5.7 mmol) in dry ether (5 mL) was added dropwise to a stirred solution of n-hexylmagnesium bromide made from the bromide (1.23 g, 7.5 mmol) and magnesium (0.18 g, 7.2 mmol) in dry ether (10 mL), and the solution was then stirred for 1 h and then quenched with saturated ammonium chloride solution. The separated organic layer was combined with the ether extracts of the aqueous phase and then dried ($MgSO_4$), and the ether was removed under vacuum. The crude product was purified by column chromatography (silica, hexane/ether (2:1)) (65%). MS (*m/z*, rel intensity): (244, 0.0, M^+), (159, 6.5, M – 85), (85, 100). Deprotection of 1-(tetrahydropyranyloxy)nonan-3-ol was achieved in the usual way (methanol, H^+) and after addition of H_2O, the solution was extracted with dichloromethane. Standard workup then provided **1,3-nonanediol (60%)** (bp 100–103 °C, 1 mm (lit.[47] bp 90–92 °C, 0.8 mm)). ^{13}C NMR: 72.22, 61.73, 38.24, 37.82, 31.79, 29.26, 25.48, 22.58, 14.03. MS (*m/z*, rel intensity): (160, 0.0, M^+), (113, 30.0), (92, 21.8), (75, 100.0), (70, 21.3), (57, 62.1), (55, 45.1), (45, 47.2), (43, 55.0), (41, 45.4), (29, 60.1).

Procedure B: 2-Nonyn-1-ol[48] was obtained by bubbling dry gaseous formaldehyde (thermolysis of paraformaldehyde at 200 °C) into an ether solution of oct-1-ynylmagnesium bromide, obtained from 1-octyne and ethylmagnesium bromide. Standard workup and distillation provided the alcohol (86%) as a colorless oil (bp 88–90 °C, 7 mm). ^1H NMR: 4.16 (2 H, s), 2.95 (1 H, br s), 2.12 (2 H, tt, J = 7 Hz, 2.3 Hz), 1.1–1.4 (8 H, m), 0.8 (3 H, t, J = 7 Hz). ^{13}C NMR: 86.03, 78.22, 50.82, 31.18, 28.45, 28.40, 22.35, 18.55, 13.81. MS (*m/z*, rel intensity): (140, 0.0 M^+), (93, 37.3), (79, 28.4); (70, 38.0), (69, 32.0), (67, 53.8). **(*E*)-2-Nonen-1-ol:**[49] Non-2-yn-1-ol (3.0 g, 21 mmol) in dry ether (50 mL) was added dropwise to a stirred solution of $LiAlH_4$ (1.63 g, 43 mmol) in dry ether (300 mL). This solution was refluxed for 36 h and then decomposed with wet ether and ice, followed by ammonium chloride solution. The ether extracts of the aqueous phase were combined, dried ($MgSO_4$), and evaporated to provide the *E*-allylic alcohol (bp 102–104 °C, 12 mm, 76%) and 1,2-nonadiene (bp 40–50 °C, 12 mm, 17%). ^1H NMR: 5.61 (2 H, m, vinylic), 4.05 (2 H, d, J = 5.8 Hz, CH_2OH), 1.95 (2 H, dt, allylic), 1.57 (1 H, br s, OH), 1.15–1.40 (8 H, m), 0.85 (3 H, t, CH_3). ^{13}C NMR: 133.53, 128.78, 63.79, 32.19, 31.69, 29.08, 28.84, 22.58, 14.05. MS (*m/z*, rel intensity): (142, 0, M^+), (95, 21.9), (82, 27.1), (68, 22.3), (67, 24.3), (57, 100), (55, 42.5).

(±)-2,3-Epoxynonan-1-ol. (*E*)-2-Nonen-1-ol (1.91 g, 13 mmol) and *m*-chloroperbenzoic acid (MCPBA) (85%; 2.87 g, 17 mmol) were stirred in dichloromethane (50 mL) for ca. 3 h after which the reaction mixture was washed thoroughly with saturated sodium carbonate solution and sodium chloride solution. The organic phase was dried ($MgSO_4$) and solvent removed under vacuum to provide a white solid, which appeared as white needles after recrystallization from hexane (mp 42–42.5 °C; 77%). Anal. Calcd for $C_9H_{18}O_2$: C, 68.3; H, 11.5. Found: C, 67.8; H, 11.6. ^{13}C NMR: 61.75, 58.53, 56.03, 31.66, 31.51, 28.99, 25.84, 22.49, 13.9. MS (*m/z*, rel intensity): (158, 0, M^+), (97, 40.0), (70, 14.2), (69, 18.8), (57, 27.4), (55, 100). The above epoxy alcohol (0.5 g, 3 mmol) was dissolved in dry THF (5 mL) and cooled to –30 °C (dry ice/CCl_4 bath). Red-Al (3.4 M in toluene, 2.6 mL, 9 mmol) was added dropwise and the reaction mixture stored at –10 °C for 2 days.[39] The reaction was then quenched (H_2O) and extracted well with ether and the combined ether extracts were washed with saturated NaCl solution, separated, and dried ($MgSO_4$) and the ether was removed under vacuum. GC analysis indicated the presence of 1,3-nonanediol only. ($LiAlH_4$ reduction produces roughly equal amounts of 1,2- and 1,3-nonanediols.) Distillation provided **(±)-1,3-nonanediol (24)** (104–105 °C, 1 mm, 75%. (lit.[47] bp 90–92 °C; 0.8 mm)), whose spectroscopic properties were identical with those described above. **Sharpless Epoxidation**[37] **of (*E*)-2-Nonen-1-ol: (–)-2,3-Epoxynonan-1-ol.** A round-bottomed flask equipped with a magnetic stirrer and serum cap assembly was flame dried under a N_2 purge and cooled to –20 °C (dry ice, carbon tetrachloride) whence dry dichloromethane (from CaH_2) was added. The following were then added sequentially via syringe (through the serum cap) to the stirred solution: titanium tetraisopropoxide (1.0 g, 3.5 mmol), L-(+)-diethyl tartrate (anhydrous; 0.73 g, 3.5 mmol), which were stirred for 5 min. The (*E*)-2-nonen-1-ol (0.5 g, 3.5 mmol) and *tert*-butyl hydroperoxide (3 M in toluene, 2.4 mL, 7 mmol) were then added and the resulting homogeneous solution was stored in the freezer (–10 °C) for 24 h. To the cooled flask (–20 °C) was added aqueous tartaric acid (10 mL of 10% solution), which caused solidification of the aqueous layer. After 30 min, the cooling bath was removed and stirring continued until the aqueous layer became clear. The organic layer was washed with H_2O and cooled (0 °C) and sodium hydroxide (10 mL of 1 M solution) was added and the whole stirred (30 min). The organic phase was washed (NaCl solution), dried ($MgSO_4$), and concentrated to provide a yellow oil, which was chromatographed on silica gel (hexane/ether 1:1) to yield (–)-2,3-epoxynonan-1-ol. Anal. Calcd for $C_9H_{18}O_2$: C, 68.3; H, 11.5. Found: C, 68.2; H, 11.5. Mp: 45–45.5 °C; $[\alpha]^{24}_D$ = 38.9° (*c* = 0.0175 in $CHCl_3$). Spectral data matched those for the raemic epoxy alcohol. **(+)-2,3-Epoxynonan-1-ol** resulted from a similar procedure except that D-(–)-diethyl tartrate was employed and

(47) Buckingham, J., Ed. *Dictionary of Organic Compounds*, 5th ed.; Chapman and Hall: New York, 1982; p 439.

(48) Raphael, R. A.; Sondheimer, F. *J. Chem. Soc.* **1950**, 2101.

(49) Raphael, R. A.; Sondheimer, F. *J. Chem. Soc.* **1950**, 3185.

matching spectra, etc., were obtained. $[\alpha]^{20}_D$ +38.7° (c = 0.0295 in $CHCl_3$). ^1H NMR: δ 3.89 (1 H, dd, J = 12.5, 2.4 Hz) and 3.61 (1 H, dd, J = 12.5, 4.2 Hz, CH_2OH), 2.93 (1 H, td, J = 5.6, 2.4 Hz, H_3), 2.89 (1 H, dt, J = 2.4, 4.2 Hz, H_2), 1.2-1.67 (m, 11 H, CH_2 and OH), 0.86 (3 H, t, J = 6.8 Hz, CH_3). (This ^1H spectrum matched those for the (±) and (-) epoxy alcohols.) **Red-Al reductions** of the (+) and (-) epoxy alcohols were performed as described for the (±) alcohol, the (-) epoxy alcohol provided (+)-nonane-1,3-diol (64%), and the (+) epoxy alcohol the (-)-nonane-1,3-diol (53%). **(+)-Nonane-1,3-diol**: $[\alpha]^{20}_D$ = +6.03 (c = 1.2 in ethanol). ^1H NMR: 3.8 (3 H, m, CH-O), 2.2 (2 H, br s, OH), 1.2-1.7 (12 H, m), 0.8 (3 H, t, J = 6.8 Hz, CH_3). The ^{13}C and MS data were essentially identical with those listed for the racemic compound. **(-)-Nonane-1,3-diol**: $[\alpha]^{20}_D$ = -5.4° (c = 1.40 in ethanol) with appropriate ^1H and ^{13}C NMR and MS.

Ozonolysis of (*R*,*Z*)-(+)-12-hydroxyoctadec-9-enoic acid was conducted in the standard way at -70 °C (methanol solvent; dimethyl sulfide present) to provide **(*R*)-3-hydroxynonanal** (Kugelrohr distillation: 120 °C, 1 mm). ^1H NMR: 4.1 (1 H, m, CH-OH), 2.6 (2 H, m, CH_2CO), 2.4 (1 H, br s, OH), 1.3 (11 H, m), 0.8 (3 H, t, CH_3). MS (m/z, rel intensity): (158, 0, M^+), (73, 28.9), (70, 36.6), (57, 24.4), (55, 66.7), (45, 54.7). **Reduction** of the above aldehyde was effected with $NaBH_4$ in methanol in the normal way to provide **(*R*)-(-)-nonane-1,3-diol** (Kugelrohr: 100 °C, 0.1 mm) (lit.[50] bp 97-99 °C, 0.1 mm)). $[\alpha]^{22}_D$ = -5.5° (c = 5.8 in ethanol) (lit.[50] $[\alpha]^{22}_D$ = -6.30° (c = 10 in ethanol)). MS (m/z, rel intensity): (113, 22.1), (97, 14.6), (75, 100), (70, 24.4), (57, 53.2), (55, 52.0), (45, 43.3).

Hydroxy-Substituted Spiroketals. The syntheses of some of the various hydroxy-substituted spiroketals have been described elsewhere[14] and will be reported in full in a separate publication.[30]

Acknowledgment. We (University of Queensland and Queensland Department of Primary Industries) are grateful to the Rural Credits Development Scheme and the Australia Research Grants Committee for partial support of this work. Useful exchanges with Dr. Brian Fletcher, Dr. Tom Bellas, and Prof. K. Mori (Tokyo) are acknowledged.

(50) Diaper, D. G. M.; Mitchell, D. L. *Can. J. Chem.* **1960**, *38*, 1976.

Diterpenes from the Marine Sponge *Aplysilla polyrhaphis* and the Dorid Nudibranch *Chromodoris norrisi*[1]

Steven C. Bobzin and D. John Faulkner*

Scripps Institution of Oceanography (A-012F), University of California, San Diego, La Jolla, California 92093

Received January 9, 1989

Nine diterpenes of the spongian class have been isolated from the marine sponge *Aplysilla polyrhaphis* collected in the Gulf of California. *A. polyrhaphis* contained the known compounds macfarlandin E (**2**), aplyviolene (**3**), norrisolide (**4**), shahamin C (**5**), the γ-lactone **6**, and four novel diterpenes, polyrhaphins A-D (**7-10**). The structures of the polyrhaphins were determined by interpretation of spectral data and chemical interconversions. Polyrhaphin D (**10**) is the first example of the isospongian carbon skeleton. Macfarlandin E (**2**), norrisolide (**4**), shahamin C (**5**), and polyrhaphin A (**7**) were also isolated from specimens of the dorid nudibranch *Chromodoris norrisi* that were collected in the same locality as *Aplysilla polyrhaphis*, which is the presumed dietary source. Shahamin C (**5**), the γ-lactone **6**, and polyrhaphin C (**9**) inhibit feeding by the Gulf of California rainbow wrasse *Thalossoma lucasanum*. Some of the diterpenes also exhibit antimicrobial activity.

We have recently undertaken an investigation of sponges within the order Dendroceratida to test the hypothesis that the absence of siliceous spicules which is characteristic of this order may be compensated by an increase in the variety and quantity of secondary metabolites produced by the animals. This hypothesis originated from the observation that the loss of a physical defense mechanism, such as spicules, may result in the development of an alternate chemical defense mechanism.[2] The purple dendroceratid sponge *Aplysilla polyrhaphis* was collected in a mangrove lagoon on Isla San Jose in the Gulf of California. This extremely fragile, leafy sponge was not overgrown by other invertebrates or algae in this highly diverse and productive environment.

Nine diterpenes derived by rearrangement of the spongian diterpene skeleton **1** have been isolated from *A. polyrhaphis*. The major metabolite, macfarlandin E (**2**), has been previously reported from the dorid nudibranch *Chromodoris macfarlandi*,[3,4] while the analogous monoacetate, aplyviolene (**3**), was isolated from the dendroceratid sponge *Chelonaplysilla violacea*.[5] Norrisolide (**4**) was originally found in the nudibranch *Chromodoris norrisi*,[6] while shahamin C (**5**) was recently reported from the sponge *Dysidea* sp.,[7] and the γ-lactone **6** was isolated from the sponge *Aplysilla rosea*.[8] Four new rearranged spongian diterpenes, polyrhaphins A (**7**), B (**8**), C (**9**), and D (**10**), have been isolated and their structures determined. Polyrhaphins A-C (**7-9**) are rearranged spongian diterpenes each consisting of a hydrocarbon portion derived from the A and B rings of the spongian diterpene skeleton **1** and an oxygenated portion derived from the C and D rings of the spongian skeleton. In determining the structures of these compounds the nature of each of these fragments was elucidated separately, and the two parts were then related to one another. Polyrhaphin D (**10**) is a novel isospongian diterpene in which a 2,3-fused tetrahydrofuran ring replaces the 3,4-fused tetrahydrofuran ring normally found in spongian diterpenes. Polyrhaphin D

(1) This research was presented at the 196th ACS National Meeting (Abstract 388) in Los Angeles, CA, September 1988.
(2) Faulkner, D. J.; Ghiselin, M. T. *Mar. Ecol. Prog. Ser.* **1983**, *13*, 295.
(3) Molinski, T. F.; Faulkner, D. J; Cun-Heng, H.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 4564.
(4) The structure of macfarlandin E (**2**) has also been reported under the name aplyviolacene,[5a] but the lack of spectral data in the communication prevented us from confirming that the compounds were identical.
(5) (a) Hambley, T. W.; Poiner, A.; Taylor, W. C. *Tetrahedron Lett.* **1986**, 3281. (b) Buckleton, J. S.; Bergquist, P. R.; Cambie, R. C.; Clark, G. R.; Karuso, P.; Rickard, C. E. F. *Acta Crystallogr.* **1986**, *C42*, 2846.
(6) Hochlowksi, J. E.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1983**, *48*, 1141.
(7) Carmely, S.; Cojocaru, M.; Loya, Y.; Kashman, Y. *J. Org. Chem.* **1988**, *53*, 4801.
(8) Karuso, P.; Taylor, W. C. *Aust. J. Chem.* **1986**, *39*, 1629.

0022-3263/89/1954-3902$01.50/0