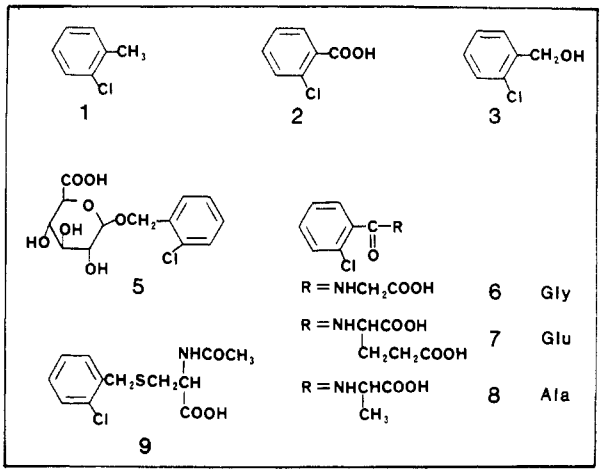

Figure 2. 2-Chlorotoluene and its metabolites from rats.

ACKNOWLEDGMENT

We thank L. E. Staiger for assistance, R. L. Carney for NMR analysis, and S. Gelfand (Occidental Chemical Corp., Hooker Industrial and Specialy Chemicals) for initiating, funding, and coordinating this research.

Registry No. 1, 95-49-8; 2, 118-91-2; 3, 17849-38-6; 5, 87039-95-0; 6, 16555-60-5; 7, 87039-96-1; 8, 87039-97-2; 9, 40379-72-4; 2-chlorobenzoic acid β-D-glucuronide, 87039-94-9.

LITERATURE CITED

Caldwell, J. In "Conjugation Reactions in Drug Biotransformation"; Aitio, A., Ed.; Elsevier/North Holland Biomedical Press: New York, 1978; p 111.
Callow, E. H.; Hele, T. S. *Biochem. J.* **1926**, *20*, 598.
Clapp, J. J.; Young, L. *Biochem. J.* **1970**, *118*, 765.
Gelfand, S. In "Kirk-Othmer Encyclopedia of Chemical Technology", 3rd ed.; Wiley: New York, 1979; Vol. 5, p 819.
Goto, J.; Suzaki, K.; Nambura, T. *Chem. Pharm. Bull.* **1979**, *27*, 1926.
Hildebrandt, H. *Beitr. Chem. Physiol. Pathol.* **1903**, *3*, 365.
Quistad, G. B.; Staiger, L. E.; Jamieson, G. C.; Schooley, D. A. *J. Agric. Food Chem.* **1982**, *30*, 895.
Quistad, G. B.; Staiger, L. E.; Jamieson, G. C.; Schooley, D. A. *J. Agric. Food Chem.* **1983**, *31*, 589.
Riley, V. *Proc. Soc. Exp. Biol. Med.* **1960**, *104*, 751.
Seutter-Berlage, F.; Rietveld, E. C.; Plate, R.; Klippert, P. J. M. *Adv. Exp. Med. Biol.* **1982**, *136A*, 359.
Vandenbergh, P. A.; Olsen, R. H.; Colaruotolo, J. F. *Appl. Environ. Microbiol.* **1981**, *42*, 737.
van Doorn, R.; Bos, R. P.; Brouns, R. M. E.; Leijdekkers, Ch.-M.; Henderson, P. Th. *Arch. Toxicol.* **1980**, *43*, 293.
van Doorn, R.; Leijdekkers, Ch.-M.; Bos, R. P.; Brouns, R. M. E.; Henderson, P. Th. *JAT, J. Appl. Toxicol.* **1981**, *1*, 236.
Wold, J. S.; Emmerson, J. L. *Pharmacologist* **1974**, *16*, 196.

Received for review May 6, 1983. Revised manuscript received July 21, 1983. Accepted August 4, 1983.

Isolation and Identification of Novel Lactones from Male Mexican Fruit Flies

Jerry B. Stokes,* Edward C. Uebel, John D. Warthen, Jr., Martin Jacobson, Judith L. Flippen-Anderson, Richard Gilardi, Leonard M. Spishakoff, and Kenneth R. Wilzer

Two isomeric γ-lactones were isolated from the male Mexican fruit fly, *Anastrepha ludens* (Loew), Diptera, Tephritidae, and characterized by ^1H and ^{13}C NMR, IR, MS, and X-ray analyses. The structures were [3a*R**-(3aα,4α,7aβ)]-4-ethenylhexahydro-4,7a-dimethyl-2(3*H*)-benzofuranone [$C_{12}H_{18}O_2$, orthorhombic, $P2_12_12_1$, a = 7.448 (6) Å, b = 8.582 (4) Å, and c = 17.860 (9) Å] and [3a*R**-(3aα,4β,7aβ)]-4-ethenylhexahydro-4,7a-dimethyl-2(3*H*)-benzofuranone [$C_{12}H_{18}O_2$, orthorhombic, $P2_12_12_1$, a = 6.389 (9) Å, b = 11.461 (15) Å, and c = 15.410 (21) Å]. X-ray data include final atomic coordinates, bond lengths, bond angles, and molecular numbering schemes. Two alcohols, (*Z*)-3-nonen-1-ol and (*Z*,*Z*)-3,6-nonadien-1-ol, were also isolated from the male Mexican fruit fly. These compounds are currently being evaluated for possible pheromonal activity.

Various fruit fly species can cause heavy production losses of cultivated varieties of fruit crops. The Mexican fruit fly, *Anastrepha ludens* (Loew), is responsible for a 10% loss in the annual citrus crop and a 5% loss to other food crops in Mexico. Although this pest is located predominantly in Mexico, trapping along the U.S.–Mexican border has shown the seasonal migration of this pest into citrus groves located in the southwestern United States (Baker et al., 1944).

Biologically Active Natural Products Laboratory (J.B.S., E.C.U., J.D.W., and M.J.) and Insect Physiology Laboratory (K.R.W.), Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Monterrey, Mexico (L.M.S.), and Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC 20375 (J.L.F.-A. and R.G.).

Currently, a protein lure is used as an attractant for assessing Mexican fruit fly infestation. However, a more potent attractant such as a sex pheromone would be advantageous for assessing or controlling infestations. Aguirre (1974) and Steer (1975) suggested that the male Mexican fruit fly attracts the female of this species by a sexual lure. With their work in mind, Gaxiola (1977) isolated four compounds from the male Mexican fruit fly. He identified two nine-carbon unsaturated alcohols, (*Z*)-3-nonen-1-ol and (*Z*,*Z*)-3,6-nonadien-1-ol, and proposed structures for two fused cyclohexyl lactones.

Our study was carried out to determine the chemical structures of these lactones from the male Mexican fruit fly.

EXPERIMENTAL SECTION

Materials. Mexican fruit fly male abdomens (317 000) in 95% ethanol were supplied by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service

(APHIS) at Monterrey, Mexico. Authentic samples of (*Z*)-3-nonen-1-ol and (*Z*,*Z*)-3,6-nonadien-1-ol were prepared by the methods of Kajiwara et al. (1975). All samples were purified by liquid or gas–liquid chromatography (LC or GLC) and the purity was determined by GLC analyses.

Extraction of Male Fruit Fly Abdomens. Male fruit flies were separated from females within a few days after eclosion. When the males were between 6 and 13 days old, their abdomens were clipped at the first or second segment and stored in 95% ethanol (ca. 1000 tips/10 mL). The following procedure was used batchwise for the initial purification of male-produced compounds from the tips. The tips of 104 000 males and the ethanol in which they were stored were transferred to a flask. The shipping jars were rinsed with hexane; the rinse was added to the flask of tips. The contents of the flask were filtered, and the hexane and ethanol layers were separated. The alcohol layer was extracted with an additional 500 mL of hexane, and the abdomens were washed with 1 L of hexane. The wash was repeated 2 times with the same amount of hexane. The hexane layers, extracts, and washes were dried over anhydrous sodium sulfate, and the solvent was removed in vacuo to yield 9.1 g of yellow oil.

Separation of Male Fruit Fly Produced Compounds. The hexane-soluble oil was chromatographed on 200 g of Florisil (60–100 mesh, 5.0 × 25 cm column), and the following fractions were collected: fraction 1, hexane (2 L); fraction 2, hexane–ethyl acetate (97:3, 2 L); fraction 3, hexane–ethyl acetate (94:6, 1 L); fraction 4, hexane–ethyl acetate (94:6, 1 L); fraction 5, hexane–ethyl acetate (90:10, 1 L); fraction 6, hexane–ethyl acetate (90:10, 1 L). The fractions were analyzed by GLC and gas chromatography–mass spectroscopy (GC–MS).

Fractions 3–5 were combined and chromatographed on three columns of silica gel (5–10 g, J. T. Baker 3404, 40–140 mesh) and then on three columns of Florisil (5–10 g, 60–100 mesh) with hexane–ethyl acetate (98:2 to 90:10) as the eluent. Florisil fractions containing the lactones were passed through Sep-PAK C_{18} cartridges (Waters Associates) with methanol–water (80:20). The same procedure was used for Florisil fractions containing the alcohols. The eluates of the lactones and the alcohols were concentrated, and the residues were dissolved in hexane and chromatographed twice on a 2-g column of 20% silver nitrate–Florisil (60–100 mesh) with hexane–ethyl acetate (95:5 to 50:50). As a final purification step, each lactone and each alcohol were chromatographed on 5 g of silica gel with hexane–ethyl acetate (90:10). OR or ORD measurements were not obtained before field testing these natural lactones. Purity of the isolated lactones was determined on a 25 m × 0.2 mm i.d. Carbowax 20M fused silica capillary column (Scientific Glass Engineering). Purity of the alcohols was determined on a 10% Carbowax 20M on 80–100 mesh Chromosorb W-AW (1.8 m × 4 mm i.d.).

Capillary GC–MS Analyses. A 10 m × 0.2 mm i.d. fused silica column coated with silicone rubber, SE-30 (Hewlett-Packard), or a 25 m × 0.2 mm i.d. fused silica column coated with Carbowax 20M (Scientific Glass Engineering) was used for GC–MS analyses. Data were collected on a Hewlett-Packard Model 5990A GC–MS equipped with a capillary interface and Model 9825A data module. Electron ionization voltage was 70 eV.

NMR Spectral Analyses. ^{1}H NMR spectra were recorded on a JEOL FX-60Q instrument at 59.75 MHz and on a Nicolet NT-360 instrument at 360 MHz. Samples were run in $CDCl_3$, and chemical shifts are reported in ppm by using the residual $CHCl_3$ peak (7.27 ppm) as an internal standard. ^{13}C NMR spectra were recorded on a JEOL FX-60Q instrument at 15.00 MHz and are completely proton decoupled. Samples were run in $CDCl_3$, and chemical shifts are reported in ppm by using the middle peak (76.9 ppm) of the $CDCl_3$ as an internal standard.

Table I. Atomic Coordinates[a] for Molecule III

	x	y	z	B_{eq}, Å^2 [b]
C(1)	0.6847 (7)	0.5437 (7)	0.5920 (3)	6.5 (3)
C(2)	0.6207 (9)	0.7068 (8)	0.6163 (4)	7.1 (4)
C(3)	0.4838 (10)	0.6982 (7)	0.6788 (3)	7.2 (4)
C(4)	0.3146 (8)	0.5970 (6)	0.6648 (3)	5.0 (2)
C(5)	0.3917 (7)	0.4413 (6)	0.6376 (2)	4.2 (2)
C(6)	0.2794 (8)	0.2993 (7)	0.6169 (3)	5.9 (3)
C(7)	0.4236 (11)	0.2000 (7)	0.5816 (3)	6.5 (3)
O(8)	0.5646 (6)	0.2846 (4)	0.5596 (2)	6.2 (3)
C(9)	0.5175 (7)	0.4500 (6)	0.5702 (3)	4.7 (2)
O(10)	0.4188 (8)	0.0609 (5)	0.5731 (3)	9.7 (5)
C(11)	0.4430 (8)	0.5007 (6)	0.4946 (2)	6.2 (3)
C(12)	0.1856 (8)	0.6796 (7)	0.6106 (3)	6.4 (3)
C(13)	0.2306 (14)	0.5609 (9)	0.7399 (4)	9.7 (5)
C(14)	0.0874 (15)	0.5743 (13)	0.7659 (6)	14.8 (7)

[a] Standard deviations, given in parentheses, are based solely on least-squares parameters. [b] The B_{eq} values are calculated according to the formula $B_{eq} = {}^4/_3 \sum_i \sum_j \beta_{ij} \bar{a}_i \bar{a}_j$, where the β's are the anisotropic thermal parameters.

IR Spectral Analyses. The IR spectra were measured as a smear or a melt on sodium chloride plates with a Perkin-Elmer Model 283 infrared spectrophotometer.

X-ray Diffraction Analyses. Both compounds III and IV crystallize in the orthorhombic space group $P2_12_12_1$ with a = 7.448 (6) Å, b = 8.582 (4) Å, and c = 17.860 (9) Å for III and a = 6.389 (9) Å, b = 11.461 (15) Å, and c = 15.410 (21) Å for IV. The quality of the crystals was not good and they sublimed during data collection. Therefore, data were collected with a very fast scan rate in 2θ (30 deg/min). In this manner a set of 895 reflections ($2\theta_{max}$ = 112°) was collected for molecule III in approximately 6.5 h and a set of 560 reflections ($2\theta_{max}$ = 90°) was collected for molecule IV in approximately 4.0 h. In both cases, the data were collected on a Nicolet P3F automatic diffractometer by using Cu Kα radiation with a graphite monochromator on the incident beam. The structures were solved by direct methods (Karle and Karle, 1966) using the MULTAN 80 system of computer programs (Main et al., 1980). The structures were refined by full-matrix least-squares methods using program ORFXLS3 (Busing et al., 1975). The function minimized was $\sum w(|F_o| - |F_c|)^2$ where the weights, w (derived from estimated standard deviations of observed intensity), were calculated according to Gilardi (1973). Hydrogen atoms were put in at calculated positions and included in the final cycles of refinement as constant parameters. Corrections for isotropic extinction were also applied during the latter stages of refinement. The final R factors (agreement between observed and calculated structure factors) were R = 7.4% and R_w = 7.6% for III and R = 8.5% and R_w = 8.8% for IV. The goodness of fit parameter (standard deviation of an observation of unit weight) at the conclusion of the refinements was 2.2 for III and 2.7 for IV. Refined coordinates for the non-hydrogen atoms are listed in Tables I and II for molecules III and IV, respectively. Coordinates of hydrogen atoms and tables of structure factors are available.

RESULTS

The structure for III (Figure 5), [3a*R**-(3aα,4α,7aβ)]-4-ethenylhexahydro-4,7a-dimethyl-2(3*H*)-benzofuranone, is supported by the following data: ^{1}H NMR ($CDCl_3$, 59.75 MHz) δ 1.05 (s, CH_3), 1.37 (s, CH_3), 1.53–2.49 (m, 9 H), 4.82, 5.05 (d, J = 18 Hz, d, J = 9.5 Hz, 2 H, CH_2=CH),

Table II. Atomic Coordinates[a] for Molecule IV

	x	y	z	B_{eq}, Å^2 [b]
C(1)	0.2152 (20)	−0.1322 (9)	0.4580 (8)	7.3 (4)
C(2)	0.2199 (24)	−0.1819 (9)	0.3650 (9)	9.1 (5)
C(3)	0.3169 (21)	−0.0944 (10)	0.2980 (7)	7.9 (4)
C(4)	0.2043 (17)	0.0247 (9)	0.2970 (8)	6.1 (3)
C(5)	0.2016 (17)	0.0629 (8)	0.3936 (6)	5.4 (2)
C(6)	0.1172 (19)	0.1817 (8)	0.4246 (6)	6.1 (3)
C(7)	0.1170 (22)	0.1615 (11)	0.5202 (8)	7.3 (4)
O(8)	0.1176 (13)	0.0452 (7)	0.5385 (4)	7.0 (4)
C(9)	0.0903 (19)	−0.0198 (9)	0.4554 (7)	5.6 (2)
O(10)	0.1150 (15)	0.2324 (7)	0.5788 (5)	8.6 (4)
C(11)	−0.1376 (19)	−0.0391 (9)	0.4459 (7)	7.2 (4)
C(12)	0.3479 (21)	0.1133 (10)	0.2466 (7)	8.5 (4)
C(13)	−0.0123 (23)	0.0285 (10)	0.2579 (8)	7.5 (4)
C(14)	−0.0832 (22)	−0.0369 (10)	0.1954 (9)	9.4 (5)

[a] Standard deviations, given in parentheses, are based solely on least-squares parameters. [b] The B_{eq} values are calculated according to the formula $B_{eq} = {}^4/_3 \sum_i \sum_j \beta_{ij} \bar{a}_i \bar{a}_j$, where the β's are the anisotropic thermal parameters.

5.71 (dd, J = 18 and 9.5 Hz, 1 H, CH=CH_2); ^{13}C NMR ($CDCl_3$, 15.00 MHz) δ 20.1, 20.2, 28.9, 30.2, 36.1, 37.1, 38.5, 55.5, 86.1, 112.9, 139.9, 175.9; mass spectrum m/z (rel intensity), 194 (M^+, 7), 179 (27), 166 (11), 161 (16), 152 (14), 151 (12), 136 (38), 135 (24), 123 (32), 108 (67), 93 (78), 81 (base peak), 79 (68), 67 (60), 55 (29), 53 (37), 43 (84), 41 (44); IR, 3040, 2940, 1765, 1632, 1260, 1220, 1195, 1018, 922 cm^{-1}.

The structure for IV (Figure 6), [3aR*-(3aα,4β,7aβ)]-4-ethenylhexahydro-4,7a-dimethyl-2(3H)-benzofuranone, is supported by the following data: ^{1}H NMR ($CDCl_3$, 59.75 MHz) δ 1.03 (s, CH_3), 1.25 (s, CH_3), 1.68–2.61 (m, 9 H), 4.97, 5.19 (d, J = 18 Hz, d, J = 9.5 Hz, 2 H, CH_2=CH), 5.92 (dd, J = 18 Hz and 9.5 Hz, 1 H, CH=CH_2); ^{13}C NMR ($CDCl_3$, 15.00 MHz) δ 16.2, 20.3, 20.8, 29.3, 37.0, 37.9, 38.3, 53.4, 85.5, 111.4, 147.7, 175.9; mass spectrum m/z (rel intensity), 194 (M^+, 4), 179 (21), 166 (17), 161 (11), 152 (9), 151 (15), 136 (17), 135 (23), 123 (23), 108 (66), 93 (64), 81 (base peak), 79 (64), 67 (52), 55 (33), 53 (34), 43 (81), 41 (43); IR, 3040, 2940, 2862, 1770, 1630, 1225, 1094, 917 cm^{-1}.

DISCUSSION

The hexane-soluble residue from the tips of the male Mexican fruit fly abdomens was chromatographed on Florisil, and each of the six fractions was analyzed by capillary GC–MS. In agreement with the findings of Gaxiola (1977), the same compounds that may be the sexual lure were concentrated in fractions 3–5. With repeated open-column chromatography on small columns of silica gel, Florisil, and silver nitrate impregnated Florisil, four compounds (I–IV) were isolated (amounts/insect: I, 10 ng; II, 5 ng; III, 82 ng; IV, 17 ng).

With capillary GC–MS, I and II were identified as C-9 unsaturated alcohols with molecular weights of 142 and 140, respectively. Gas chromatographic retention times, mass spectral, and infrared data of I and II were identical with authentic samples of (*Z*)-3-nonen-1-ol and (*Z*,*Z*)-3,6-nonadien-1-ol (Kajiwara et al., 1975).

In similar work with the closely related Caribbean fruit fly, *Anastrepha suspensa* (Loew), Nation (1975) isolated two C-9 alcohols. Although final structures were not proposed, Nation (1975) hydrogenated the isolated alcohols and converted each into a compound that had an identical retention time with that of 1-nonanol. Mass spectral data also supported C-9 alcohol structures. Nation (1975) suggested that one alcohol was monounsaturated and the other was diunsaturated, but he did not assign the positions of unsaturation. Gaxiola (1977) compared the alcohols isolated from the Mexican fruit fly with the alcohols that Nation (1975) actually isolated from the Caribbean fruit fly. Finding them to have identical gas chromatographic properties, Gaxiola (1977) suggested that they were the same.

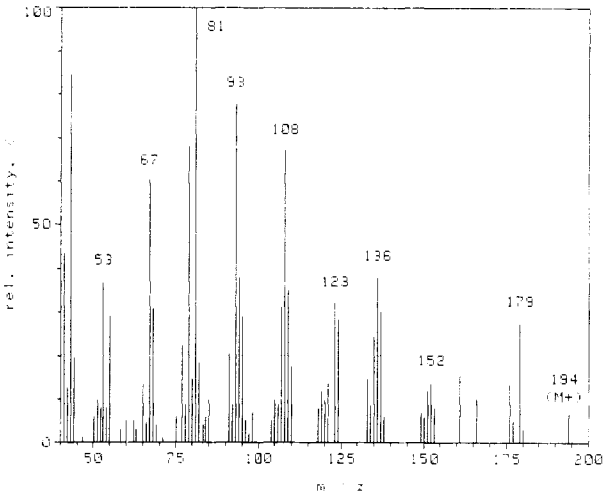

Figure 1. Mass spectrum of III.

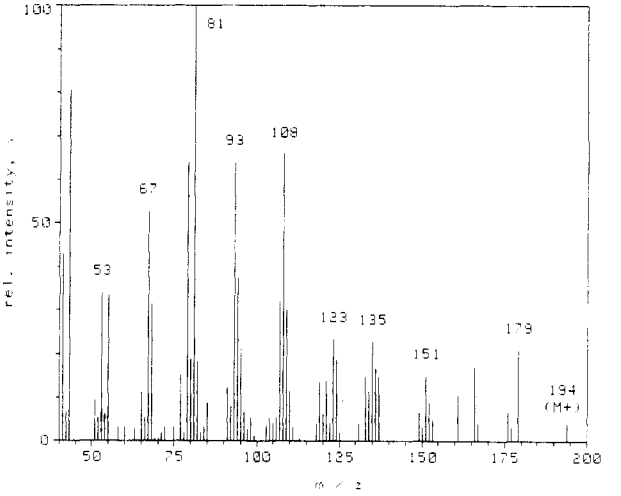

Figure 2. Mass spectrum of IV.

Two additional compounds in the Caribbean fruit fly were found and shown by Nation (1975) to be identical with each other by mass spectrometry. He reported that each had a molecular weight of 196 and an empirical formula of $C_{11}H_{16}O_3$. The infrared data suggested that these compounds were lactones, but no structures were proposed. However, Gaxiola (1977) isolated two lactones from the Mexican fruit fly, but each of these had a molecular weight of 194 and an empirical formula of $C_{12}H_{18}O_2$. The IR, NMR, and mass spectral data of the lactones that Nation (1975) actually isolated from the Caribbean fruit fly were identical with those data of the lactones that Gaxiola (1977) isolated from the Mexican fruit fly. Gaxiola (1977) was not able to come to any conclusions as to the actual structure of each lactone.

In our studies with the Mexican fruit fly lactones, the IR, NMR, and mass spectral data were comparable to those obtained by Gaxiola (1977). The mass spectra for III (Figure 1) and IV (Figure 2) have similar fragmentation patterns when electron ionization mass spectrometry is used. A molecular ion at m/e 194, a base peak at m/e 81, and an initial M − 15 (loss of methyl) were characteristic of both lactones. The presence of other ions of similar relative intensities strongly suggested that III and IV were isomeric. Empirical formulas of $C_{12}H_{18}O_2$ were assigned

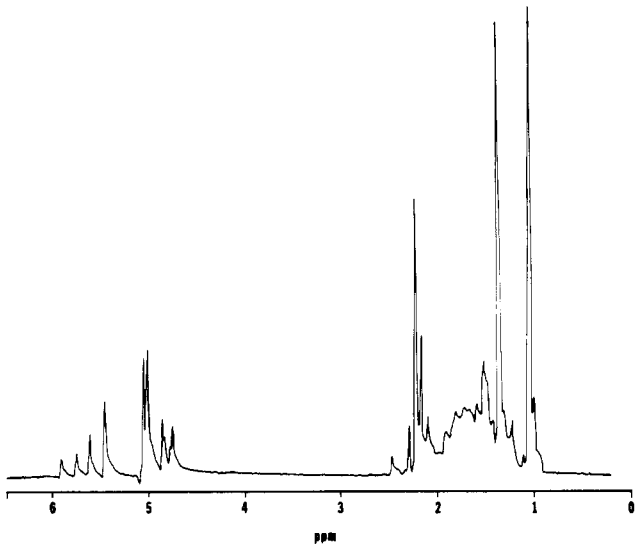

Figure 3. 59.75-MHz ^1H NMR spectrum of III.

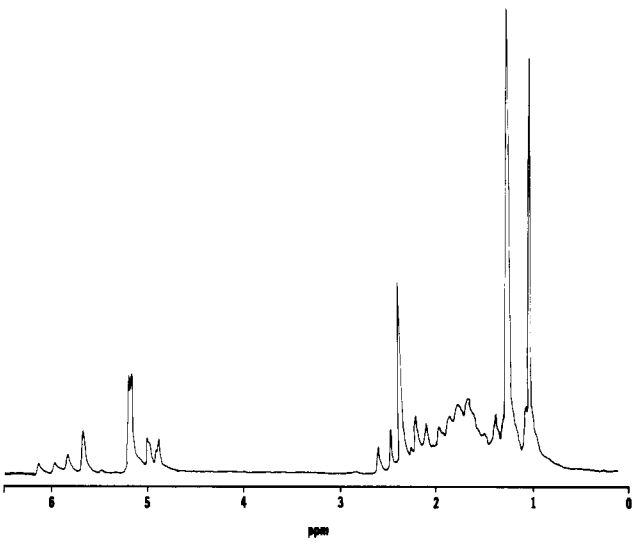

Figure 4. 59.75-MHz ^1H NMR spectrum of IV.

to the lactones, III and IV, of the male Mexican fruit fly.

The IR spectrum showed a strong C=O stretching frequency at 1780 cm^{-1} for III and 1770 cm^{-1} for IV, characteristic of that observed for γ-lactones (1760–1790 cm^{-1}) (Silverstein et al., 1974). IR data for each molecule showed similar absorptions for a vinyl C–H stretching frequency at 3040 cm^{-1}, a C=C stretching frequency at 1630 cm^{-1}, and an olefinic C–H bending frequency at about 920 cm^{-1} (Silverstein et al., 1974). The similarity of the IR spectra of these lactones also supported the assumption that III and IV were isomeric.

The ^1H NMR spectrum of III (Figure 3) showed signals at δ 4.78–5.94, characteristic of a vinyl group (Silverstein et al., 1974) while IV (Figure 4) showed a similar downfield pattern at δ 4.87–6.14. Hydrogenation of III over Pt_2O and subsequent NMR analysis of the product showed the disappearance of the signals for the olefinic protons and the appearance of new signals for an ethyl group. Thus, it was assumed that the only unsaturation was a vinyl group.

The presence of unsplit methyls at δ 1.03 and 1.25 (III) and at δ 1.05 and 1.37 (IV) suggested a *gem*-dimethyl group, but the signals for the second methyls at δ 1.25 for III and at δ 1.37 for IV were shifted downfield too much to support this *gem*-dimethyl group. The IR spectra of III and IV also did not show a doublet for a *gem*-dimethyl group at 1360–1390 cm^{-1} (Silverstein et al., 1974). On the basis of the NMR shifts of the upfield methyl and the absence of any obvious coupling with the methine proton on the vinyl in either molecule, it was concluded that this methyl and vinyl had to be situated on the same carbon. Integration of the signals for assignment of the remaining nine protons showed complex coupling with three of the protons at δ 2.11–2.49 for III and δ 2.10–2.61 for IV, while the other six protons showed signals at δ 1.3–2.0 for III and IV.

Table III. Carbon-13 NMR Chemical Shifts[a-c]

	III	IV
C(1)	29.3	30.2
C(2)	20.8**	28.9
C(3)	38.3*	38.5*
C(4)	37.9*	37.1*
C(5)	37.0*	36.1*
C(6)	53.4	55.5
C(7)	175.9	175.9
C(9)	85.5	86.1
C(11)	16.2	20.1**
C(12)	20.3**	20.2**
C(13)	147.7	139.9
C(14)	111.4	112.9

[a] In ppm from the middle peak (76.9 ppm) of the $CDCl_3$ as an internal standard. For numbering, see Figures 7 and 8. [b] Completely proton decoupled. [c] Chemical shifts with the same number of asterisks cannot be assigned with a high degree of certainty and may be interchanged within the same structure.

If the unknowns were γ-lactones, then the two methylene protons adjacent to the carbonyl would be expected to be in the δ 2.0–2.5 region. The spectrum of III at 360 MHz clearly showed coupling between the methylene protons and the methine hydrogen at the adjacent ring junction; three quartets, one with almost complete merging of the two middle signals, are present. In III, one methylene proton at δ 2.38 is coupled with the bridgehead hydrogen at δ 2.11 with a coupling constant of 14.8 Hz; likewise, the other methylene proton at δ 2.27 is coupled with this same methine but with a smaller coupling constant of 6.4 Hz. The methylene protons are coupled to each other with a coupling constant of 16.4 Hz.

Similar coupling between the methylene and the bridgehead protons has been observed for a trans-fused cyclohexyl γ-lactone (Hoye and Kurth, 1978). In addition, a shift of δ 1.34 was assigned to a methyl located at the C–O ring junction of the γ-lactone. This is comparable to methyl shifts for III at δ 1.25 and IV at δ 1.37. Thus, the structures for III and IV were trisubstituted (dimethyl and vinyl) γ-lactones. The downfield methyl (III, δ 1.25, and IV, δ 1.37) was assigned at the C–O ring junction, while the remaining methyl (III, δ 1.03, and IV, δ 1.05) and vinyl were assigned to the same carbon in the cyclohexane ring. The proton shifts of the methyl at the C–O ring junction and the IR C=O stretch support a trans-fused cyclohexyl γ-lactone for III and IV, similar to the trans-fused, synthetic lactones reported by Hoye and Kurth (1978).

The ^{13}C NMR chemical shifts of III and IV (Table III) showed the presence of 12 carbons. Shifts for a carbonyl (III, δ 175.9; IV, 175.9) correspond to that expected for five-membered lactones (Levy and Nelson, 1972). Likewise, the data supported the vinyl assignment with shifts at δ 111.4 and 147.4 for III and δ 112.9 and 139.9 for IV. Thus, on the basis of ^1H and ^{13}C NMR data and an empirical formula of $C_{12}H_{18}O_2$, we assigned the backbone ring structure of a fused cyclohexyl lactone to each molecule.

The results of the X-ray analyses on III and IV are shown in Figures 5 and 6, respectively. These figures were

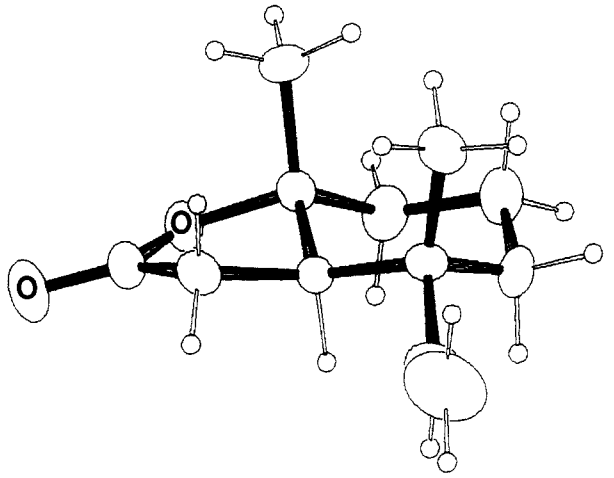

Figure 5. X-ray structure of III. The drawing is based on the final refined coordinates and thermal parameters.

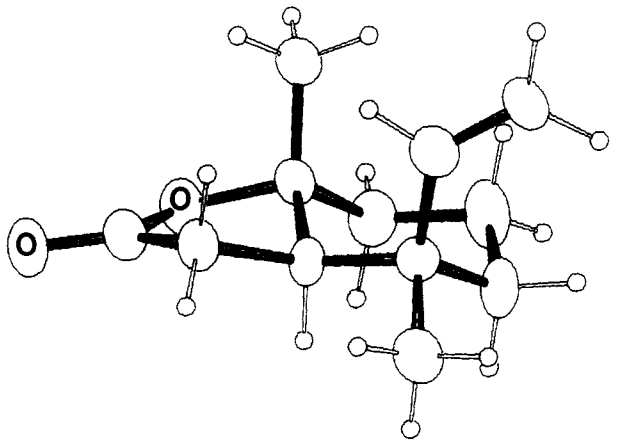

Figure 6. X-ray structure of IV. The drawing is based on the final refined coordinates and thermal parameters.

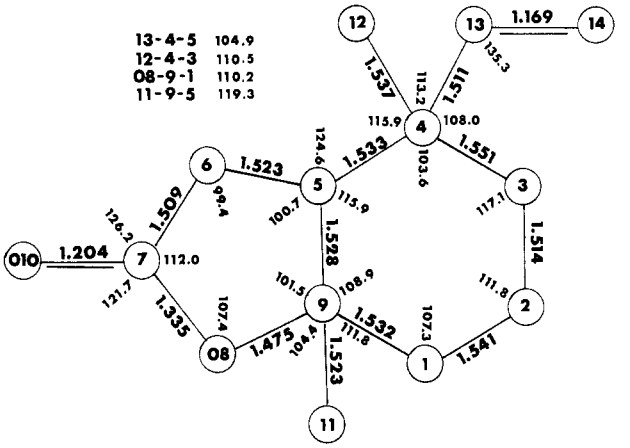

Figure 7. Bond lengths and angles for III. Based solely on the least-squares results, the standard deviations are on the order of 0.013 Å for bond lengths and 0.9° for bond angles.

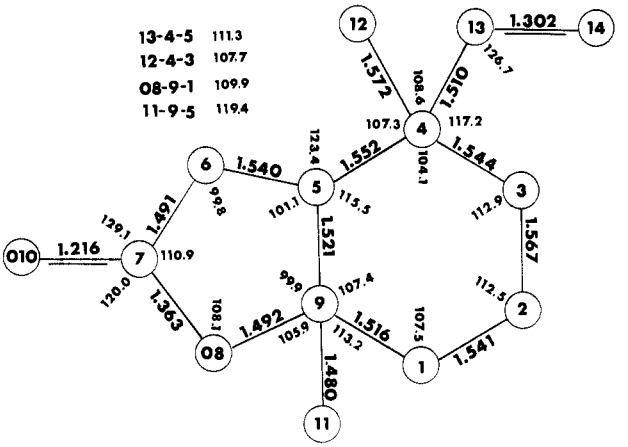

Figure 8. Bond lengths and angles for IV. Based solely on the least-squares results, the standard deviations are on the order of 0.014 Å for bond lengths and 0.9° for bond angles.

drawn by computer (program ORTEP) (Johnson, 1971) from the final refined coordinates. The two molecules have essentially the same configuration. The six-membered rings have normal chair conformations and the five-membered rings are in the envelope conformation with C(9) out of the plane in both molecules. The ring fusion is trans in both cases as indicated by the H(5)–C(5)–C(9)–C(11) torsion angle of −176.0° in III and −178.6° in IV. The only significant difference in configuration between the two molecules is at the vinyl group. In III, the C(14) of the vinyl moiety is essentially coplanar with the plane of C-(12)–C(4)–C(13) [C(12)–C(4)–C(13)–C(14) torsion angle is 4.3°] while in IV, C(14) is rotated approximately 90° out of the plane [C(12)–C(4)–C(13)–C(14) torsion angle is 91.4°]. Bond lengths and angles for molecules III and IV are shown in Figures 7 and 8, respectively.

For the most part, these values fall within expected limits of normal covalent bond lengths and angles. The estimated standard deviations (Figures 7 and 8) are measures of the precision of the diffraction experiment, assuming only random errors of measurement. With unstable crystals such as these, and rapid data collection, deviations of 4–5 ESD's (0.06–0.07 Å for distances) would not be considered unusual. Even so, the bond distance and angle observed in the vinyl group of III are anomalous. The C=C distance is only 1.169 (25) Å in III, while it is 1.302 (24) Å (a normal value) in IV. In addition, the C–C–C angle is 135.3 (9)° in III, while it is 126.7 (9)° in IV. It is extremely unlikely that the vinyl geometry in III could be so perturbed by intra- or intermolecular bonding effects; therefore, the observed values strongly suggest the occasional presence of a cocrystallized impurity or some localized deterioration of III due to X-irradiation.

The packing of the molecules in the unit cell is influenced only by van der Waals forces. There are no intermolecular contacts less than the sum of the van der Waals radii.

We have established the structure and relative configuration of the γ-lactones in the male Mexican fruit fly as III and IV. Whether a blend of these two γ-lactones and the two C-9 alcohols, I and II, is the male sex pheromone of the Mexican fruit fly is yet to be answered. Various combinations of these four compounds are currently undergoing biological evaluation.

ACKNOWLEDGMENT

We especially thank Ma. del Rosario Castanon, Nelly Alvarado, Gregorio Galindo, Juan Davila, and Salvador Alfaro, APHIS, Monterrey, Mexico, for collection of insect male abdominal tips, Dr. Robert Highet, NHLBI, NIH, Bethesda, MD, for the 360-MHz NMR spectrum of lactone III, and Dr. Nobel Wakabayashi, BANPL, ARS, USDA, Beltsville, MD, for his guidance with the interpretation of the ^{1}H and ^{13}C NMR spectra.

Registry No. III, 86852-65-5; IV, 86852-66-6; (*Z*)-3-nonen-1-ol, 10340-23-5; (*Z*,*Z*)-3,6-nonadien-1-ol, 53046-97-2.

LITERATURE CITED

Aguirre, L. A. Thesis (in Spanish), Technical University of Monterrey-Mexico, 1974.

Baker, A. C.; Stone, W. E.; Plummer, C. C.; McPhail, M. *Misc. Publ.—U.S., Dep. Agric.* **1944**, *No. 531*, 155.
Busing, W. R.; Martin, K. O.; Levy, H. A.; Ellison, R. D.; Hamilton, W. C.; Ibers, J. A.; Johnson, C. K.; Thiessen, W. E. "ORXFLS3"; Oak Ridge National Laboratory: Oak Ridge, TN, 1975.
Gaxiola, R. E. Thesis (in Spanish), Technical University of Monterrey-Mexico, 1977.
Gilardi, R. D. *Acta Crystallogr., Sect. B* **1973**, *B29*, 2089.
Hoye, T. R.; Kurth, J. *J. Org. Chem.* **1978**, *43*, 3693.
Johnson, C. K. "ORTEP-II"; Oak Ridge National Laboratory: Oak Ridge, TN, 1971; Report ORNL-3794.
Kajiwara, T.; Odake, Y.; Hatanaka, A. *Agric. Biol. Chem.* **1975**, *39*, 1617.
Karle, J.; Karle, I. L. *Acta Crystallogr.* **1966**, *21*, 849.
Levy, G. C.; Nelson, G. L. In "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists"; Wiley-Interscience: New York, 1972; p 119.
Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M. "MULTAN 80" (a system of computer programs for the automatic solution of crystal structures from X-ray diffraction data); University of York, York, England, and University of Louvain, Louvain, Belgium, 1980.
Nation, J. L. *Environ. Entomol.* **1975**, *4*, 27–30.
Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. "Spectrometric Identification of Organic Compounds", 3rd ed.; Wiley: New York, 1974.
Steer, D. A. Thesis (in Spanish), Technical University of Monterrey-Mexico, 1975.

Received for review October 25, 1982. Revised manuscript received March 10, 1983. Accepted June 28, 1983. Presented in part at the 16th Middle Atlantic Regional Meeting, American Chemical Society, Newark, DE, April 21–23, 1982. Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature.

Volatile Components of *Acacia* sp. Blossoms

Robert A. Flath,* T. Richard Mon, Gabrielle Lorenz, C. James Whitten, and James W. Mackley

Volatile component mixtures from the blossoms of three different *Acacia* sp. were examined and compared by capillary gas chromatography/mass spectrometry. Blossoms of *Acacia berlandieri* Benth. and *Acacia rigidula* Benth. are attractive to the screwworm fly *Cochliomyia hominivorax* (Coquerel), while those of *Acacia farnesiana* (L.) Willd. are inactive. A total of 114 compounds was identified in the three concentrates. Fourteen of these were found in concentrates from the two active species but could not be detected in the volatile concentrate from *A. farnesiana* blossoms. They include *trans*-5-ethenyltetrahydro-α,α,5-trimethyl-2-furanmethanol (linalool oxide A), 2-phenylethanol, *trans,cis*-2,6-nonadien-1-ol, *cis*-6-ethenyltetrahydro-2,2,6-trimethyl-2*H*-pyran-3-ol (linalool oxide D), 1-nonanol, 1*H*-indole, *trans,trans*-2,4-nonadienal, eugenol, benzyl 2-methylbutyrate, jasmone, geranylacetone, *cis*-3-hexenyl benzoate, hexyl benzoate, and benzyl salicylate.

The screwworm fly *Cochliomyia hominovorax* (Coquerel) is a serious livestock pest in the southern United States, Mexico, Central America, and South America. The female lays eggs near wounds of injured animals, and emerging maggots migrate to the wound, where they feed on living tissue, enlarging the wound and preventing healing. Mass sterile fly releases and dispersal of air-dropped baited traps are the major control strategems.

During a field study of screwworm fly behavior in southern Texas, Guillot et al. (1978) observed aggregations of screwworm flies around the blossoms of certain *Acacia* species. *Acacia berlandieri*, *Acacia rigidula*, and *Acacia greggii* shrubs contained relatively high concentrations of screwworm flies of both sexes. This was in marked contrast with that observed with *Acacia farnesiana* shrubs, which did not have any appreciable screwworm fly populations among their blossoming branches.

When blossoms of attractive species are collected, frozen for storage, and rethawed, they remain attractive to the screwworm fly (Mackley, 1978).

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California (R.A.F., T.R.M., and G.L.), Metabolism and Radiation Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota (C.J.W.), and Screwworm Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Tuxtla Gutierrez, Mexico (J.W.M.).

The present authors initiated a comparative study of blossom volatiles from several attractive and nonattractive species of *Acacia*, in an attempt to identify those constituents responsible for the observed screwworm fly attraction.

Because of its importance in the perfume industry, *A. farnesiana* Willd. has been studied in some detail by earlier workers. The most extensive modern study was done by Demole, Enggist, and Stoll of Firminich et Cie, Geneva (Demole et al., 1969; Demole and Enggist, 1969). They used an absolute of Egyptian *A. farnesiana* blossoms as their starting material and identified 38 new constituents. Guenther (1952) has reviewed the findings of earlier studies.

EXPERIMENTAL SECTION

Starting Materials. Blossoms of *A. farnesiana* (L.) Willd., *A. berlandieri* Benth., and *A. rigidula* Benth. were collected in the Mission, TX, area in 1978 and 1981 (*A. greggii* was not available in sufficient quantity for study). The blossoms were frozen, shipped to Albany, CA, in packages containing solid carbon dioxide, and held at −30 °C until used.

Vacuum Steam Distillation/Solvent Extraction. Volatile blossom components were concentrated by using a modified Likens and Nickerson head (Flath and Forrey, 1977). Heptane (Burdick & Jackson) was used as the extracting solvent, and a system pressure of 40 mmHg was maintained during the 4-h concentration interval. Yields (based on fresh weight of blossom samples) were as follows: