Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10202–10205, November 1993
Physiology

Sex pheromone for the brownbanded cockroach is an unusual dialkyl-substituted α-pyrone

(*Supella longipalpa*/electroantennogram/behavioral responses)

RALPH E. CHARLTON*†, FRANCIS X. WEBSTER‡, AIJUN ZHANG‡, COBY SCHAL§¶, DANGSHENG LIANG§, ISABELLE SRENG‖, AND WENDELL L. ROELOFS*,**

*Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456; ‡Department of Chemistry, State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210; §Department of Entomology, Cook College, Rutgers University, New Brunswick, NJ 08903; and ‖Centre National de la Recherche Scientifique, Marseille, Cedex 09, France

Contributed by Wendell L. Roelofs, July 26, 1993

ABSTRACT **Female brownbanded cockroaches, *Supella longipalpa*, emit a sex pheromone that attracts males from a distance. This pheromone was isolated and identified as 5-(2,4-dimethylheptanyl)-3-methyl-2*H*-pyran-2-one (which we refer to as supellapyrone), and its structure was confirmed by synthesis. A racemic blend of the synthetic compound elicited behavioral and electrophysiological responses comparable to the natural pheromone across a range of doses. This compound is not only a very different type of cockroach pheromone but also makes up an additional class of natural products—namely, 3,5-dialkyl-substituted α-pyrones.**

Cockroaches rely on pheromones for many aspects of their reproductive behaviors, including bringing the sexes together for mating and mediating important courtship interactions (1). Their sex pheromones can be divided into two broad classes: cuticular compounds that elicit sexual responses only after the sexes contact each other (2, 3) and volatile compounds that act over a distance. In some species the male produces the long-range sex pheromone that attracts the female (4), but as a rule, it is the females that do the luring (1). Despite the fact that a number of cockroaches rank among the most serious pests of humans and that their pheromones could have real utility in management programs, the structures of only three volatile, female-produced compounds have thus far been conclusively identified. Moreover, these pheromones are derived from just two congeneric species, *Periplaneta americana* and *Periplaneta japonica* (Orthoptera: Blattidae). Persoons *et al.* (5) identified the first cockroach pheromone component from *P. americana* and named it periplanone-B. A second, structurally related compound, periplanone-A, was also identified from *P. americana* (6, 7), whereas a similar structure (periplanone-J) has been proposed as the pheromone of *P. japonica* (8).

The brownbanded cockroach, *Supella longipalpa* (Orthoptera: Blattellidae) is a serious household pest found worldwide. The existence of a volatile female-derived sex pheromone for this species has been recognized for over 15 years (9). Females exhibit a characteristic pheromone-releasing stance (10), and Smith and Schal (11) showed that females emit a volatile pheromone only during such calling bouts; males will walk upwind from a distance of at least several meters to calling females (12). To determine the site of *S. longipalpa* pheromone production, Schal *et al.* (13) assayed male behavioral and electrophysiological responses to different female body parts. Their study revealed that the pheromone is restricted to the abdominal tergites and that, in olfactometer assays, males were most responsive to solvent extracts of the fourth and fifth tergites. Microscopic examination of the tergites showed that their surface is stippled with pores and that the density of these pores is highest on the lateral margins of tergites four and five. Each pore is connected via a long subcutaneous duct to modified epidermal cells. It is hypothesized that the ducts serve to transport the pheromone from the glandular cells to the cuticular surface where it is released into the air.

Extraction of the fourth and fifth tergites produced enough material for the isolation and identification of the potent sex pheromone. This compound, 5-(2,4-dimethylheptanyl)-3-methyl-2*H*-pyran-2-one, for which we propose the name supellapyrone, is structurally very different from the germacrane and germacrene derivatives that make up the identified periplanones. The 3,5-dialkyl-substituted pyrone not only is unusual as a component in an insect communication system but also appears to belong to an additional class of compounds from any natural source.

MATERIALS AND METHODS

Pheromone Extraction and Purification. The fourth and fifth abdominal tergites were dissected from individual 6- to 10-day-old females and collectively (150–400 female equivalents) extracted in 5–10 ml of redistilled hexane. Hexane extracts were concentrated under N_2 to ≈200 μl. The concentrate was applied to the top of a prewetted (hexane) mini-column filled with 500 mg of silica gel (100- to 200-mesh Unisil; Clarkson Chromatography Products, South Williamsport, PA) and eluted successively with redistilled hexane (2 ml), 10%, 20%, and 50% (vol/vol) CH_2Cl_2 in hexane (1 ml of each), ethyl acetate (1 ml), and methanol (2 ml).

Analytical Methods. Packed-column GC analyses were performed on a 2 m × 5 mm i.d. OV-101 (3% on 100- to 120-mesh Gas Chrom Q; Applied Science Laboratories, State College, PA). Capillary GC analyses used a 30 m × 0.25 mm i.d. Carbowax column (0.25-μm film; Econ-Cap, Alltech Associates); the temperature program was 80°C for 2 min, then 5°C/min to 220°C, and 220°C for 20 min. Fractions eluting from the GC column were collected in chilled capillary tubes. The vapor-phase Fourier transform IR (FTIR) spectrum was obtained using a gas chromatograph (Hewlett–Packard 5890) coupled to a light pipe installed in a FTIR spectrometer (Nicolet model 205XC) (14). The purified pheromone (95 ng in 10 μl of hexane) was injected on a 20 m × 0.18 mm i.d. DB-1 column (0.40-μm film; J & W Scientific, Rancho Cordova, CA). High-resolution GC-MS analyses

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EAG, electroantennogram; FTIR, Fourier transform IR; EI, electron impact.

†Present address: Department of Entomology, Kansas State University, Manhattan, KS 66506.
¶Present address: Department of Entomology, North Carolina State University, Raleigh, NC 27695.
**To whom reprint requests should be addressed.

were performed on a Kratos Analytical Instruments model MS890MS tandem mass spectrometer linked to a Hewlett–Packard 5890 gas chromatograph using electron impact (EI)-MS at 70 eV (1 eV = 1.602×10^{-19} J). Chemical ionization-MS was performed on a Finnigan-MAT (San Jose, CA) 8230 high-resolution magnetic mass spectrometer using an SS300 data system. The analytical column was a 25 m × 0.32 mm DB-1 (0.25-μm film). NMR spectra were recorded on Bruker 300 and 600 AMX NMR spectrometers; the deuterobenzene (100% deuterium) was obtained from MSD Isotopes. The UV spectrum was recorded on a Varian DMS 100 UV spectrophotometer using a 1-cm cell and absolute ethanol (1 μg/ml). Kovat's retention indices (15) for supellapyrone and its analogues were obtained on polar (30 m × 0.25 mm i.d. Carbowax column, 0.25-μm film; temperature program: 80°C for 2 min, then 10°C/min to 240°C, and 240°C for 20 min; helium carrier gas at 25 cm/sec) and nonpolar (30 m × 0.25 mm i.d. SE-30 column, 0.25 μm; 80°C for 2 min, then 8°C/min to 230°C, and 230°C for 20 min; He at 26 cm/sec) fused silica columns.

RESULTS

Initial purification of concentrated crude hexane extracts of female tergites was by silica gel column chromatography. The column was eluted with solvents of increasing polarity, and pheromone activity resided almost exclusively in the ethyl acetate fraction. Biological activity was established using a two-choice olfactometer assay in which male response to 1/1000th of a pheromone extract from a single female was readily detectable (16). For routine monitoring of active fractions, electroantennograms (EAGs) were recorded. The EAG provides a quick, sensitive screening technique (17) whose responses correlate well with male upwind taxis in olfactometer assays (12). The active fraction was concentrated and further purified by preparative GC using a 3% OV-101 nonpolar column on which the active material eluted at the same retention time as octadecane and periplanone-B. Additional purification on a polar capillary column revealed that behavioral and electrophysiological activity coincided with a single major peak whose Kovat's retention index was 2361. The retention index of periplanone-B under the same conditions was 2619, so clearly it was not that pheromone. Furthermore, *S. longipalpa* males did not respond behaviorally or electrophysiologically (by EAG) to periplanone-B. The tergites of ≈12,000 females were used to yield ≈5 μg of purified pheromone.

The active compound in this fraction was identified by FTIR, GC-MS, UV, and NMR spectroscopy. The vapor-phase IR spectrum obtained with about 95 ng of pure pheromone showed a strong absorption at 1752 cm^{-1} (C=O) and hydrocarbon absorption bands but few other features. The EI-MS spectrum gave a parent ion at m/z 236 and a base peak at m/z 124; other notable fragments included 221 (M − 15), 193 (M − 43), 165 (M − 71), 123, 125, 95, 71, and 57. The assignment of m/z 236 as the molecular ion was confirmed by chemical ionization with isobutane (237, base peak). The molecular formula was deduced as $C_{15}H_{24}O_2$ on the basis of its high-resolution mass spectrum (M^+, 236.1783; calculated, 236.1776).

The isolated compound gave two absorption maxima (λ_{max}) in the UV spectrum at 228 nm and 296 nm with molar extinction coefficients (ε_{max}) of 6844 and 15,800, respectively. This spectrum coupled with the presence of a major peak at m/z 95 in the EI-MS spectrum suggested (among other possibilities) a derivative of 2*H*-pyran-2-one (18). The 2*H*-pyran-2-one ring system requires all four unsaturation equivalents as well as the two oxygen atoms from our formula of $C_{15}H_{24}O_2$, thus leaving one or more saturated hydrocarbon side chains to account for the remaining $C_{10}H_{21}$. High-resolution EI-MS of the m/z 124 ion indicated that its molecular formula was $C_7H_8O_2$, which is again consistent with a 2*H*-pyran-2-one ring retaining two methyl groups. Further elucidation of the structure depended on NMR spectrometry; with only about 5 μg of compound, we were able to obtain a 600-MHz ^1H spectrum, a two-dimensional correlated spectrum (19), and a two-dimensional total correlation spectrum (20).

For NMR, the sample was collected in a capillary tube as it was eluted from a packed GC column (21), the capillary tube was rinsed with about 75 μl of deuterobenzene into a 5-mm thick-walled NMR tube, and the tube was sealed. The

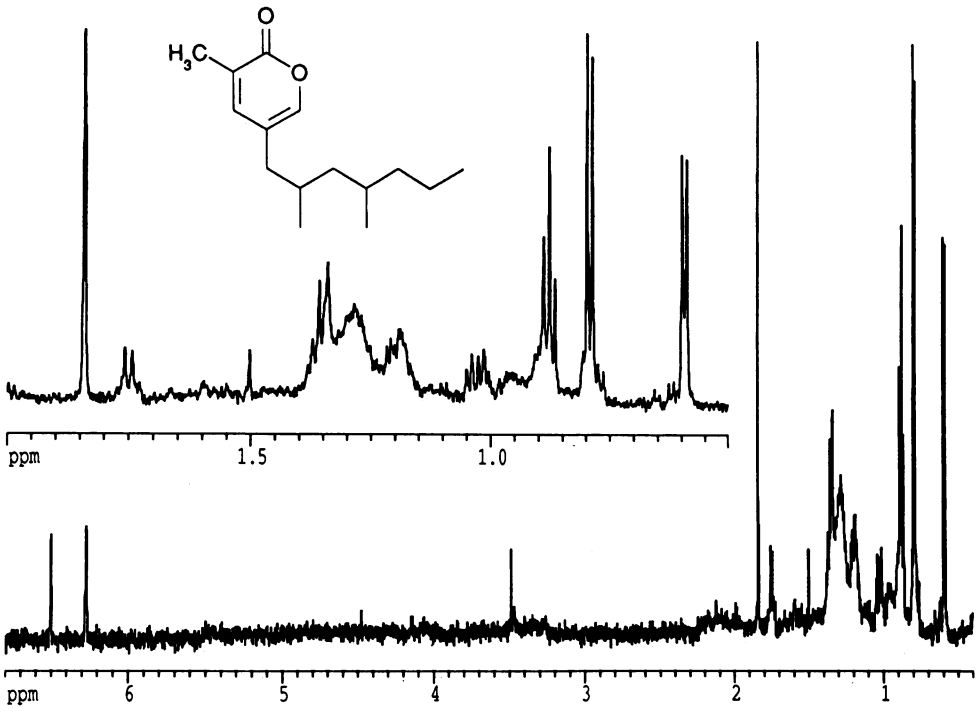

FIG. 1. ^1H NMR spectrum (600 MHz) and structure of supellapyrone.

600-MHz ^{1}H NMR spectrum (Fig. 1) revealed two downfield signals at δ 6.58 and 6.35, each corresponding to one proton; these signals represent two ring protons of an α-pyrone system. These two protons show long-range coupling (≈1.5 Hz) to each other. In addition, the signal at δ 6.58 shows further long-distance coupling to a methyl group. Also clearly visible in the spectrum are signals corresponding to four distinct methyl groups: a doublet at δ 1.91 (long-range coupling, 1.5 Hz, 3H), a triplet at δ 0.95 (8 Hz, 3H), and a doublet at δ 0.66 (8 Hz, 3H). This information led us to propose four different disubstituted α-pyrones whose structures are shown in Fig. 2. Chemical shift positions of the ring protons favored the 3,5-disubstituted structures (18), and long-range coupling data favored the 3-alkyl-5-methyl isomer.

Because the NMR data alone could not distinguish among the four possibilities, we undertook the synthesis (Fig. 3) of 3,5-dimethyl-2*H*-pyran-2-one (R = CH_3 in the scheme in Fig. 3). The key step in making the 3,5-dimethyl or 3,5-dialkyl α-pyrones, in general, is the orthoester Claisen rearrangement (22) of the appropriate allylic alcohol with triethylorthopropionate to give an ethyl ester having the correct carbon skeleton (Fig. 3). Several functional group modification steps were required to complete the synthesis, the details of which will be reported elsewhere. To our knowledge, this synthesis represents the first practical and flexible synthesis of 3,5-dialkyl-2*H*-pyran-2-ones. The only other reported synthesis of 3,5-dimethyl-2*H*-pyran-2-one (18) involved a low-yield (≈1%) photochemical decomposition.

Careful analysis of the ^{1}H NMR spectrum, the nuclear Overhauser effect difference spectrum (23), and the two-dimensional correlation spectroscopy via long-range coupling (COLOC) spectrum (24) of 3,5-dimethyl-2*H*-pyran-2-one confirmed that our original assignment of the pheromone as an alkyl-substituted α-pyrone was correct. However, the ring methyl is located at the 3 position, which is contrary to what the long-range coupling data suggested. Thus, the methyl group protons in the 3 position are coupled through six bonds to the proton at the 6 position. This unusual and unexpected coupling pattern was seen in all of the 3-methyl derivatives of α-pyrone that we have synthesized.

The remaining structural feature to be deciphered was the alkyl group located at the 5 position having a formula of C_9H_{19}. This saturated alkyl group consists of three methyl groups, which from their multiplicities can be further elaborated into two -$CHCH_3$ fragments and one -CH_2CH_3 moiety. By inference, the remaining pieces are three CH_2 groups. The correlated spectroscopy and total correlation spectroscopy experiments eliminated all but four constitutional isomers, which are shown in Fig. 3. Three of the structures (**a**, **b**, and **c** in Fig. 3) have two chiral centers and, therefore, exist as

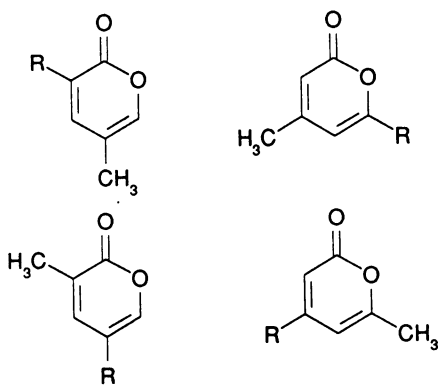

FIG. 2. Partial candidate structures of supellapyrone.

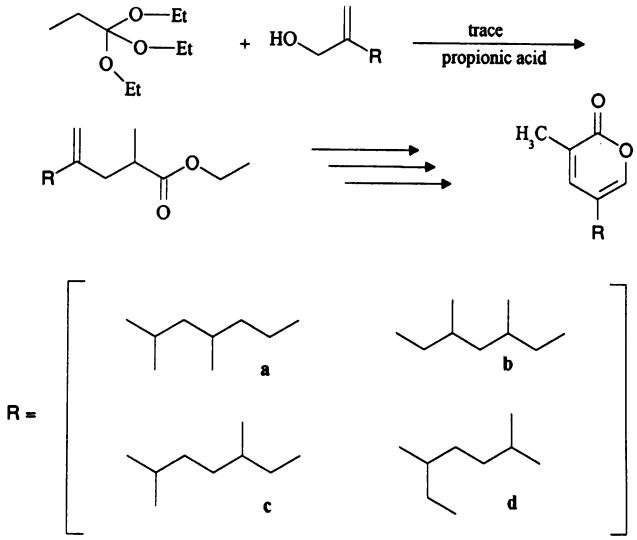

FIG. 3. Synthetic scheme for 3,5-dialkyl-substituted α-pyrones, including supellapyrone, 5-(2,4-dimethylheptanyl)-3-methyl-2*H*-pyran-2-one (**a**), and three analogues (**b**, **c**, and **d**).

four stereoisomers, whereas the remaining structure (**d** in Fig. 3) consists of two enantiomers.

To identify the pheromone, we synthesized all four sets of compounds by modifying the plan outlined in the scheme in Fig. 3. A series of allylic alcohols each having a different alkyl (R) group were prepared and are listed in Fig. 3. In addition to synthesizing the desired compound, we prepared the other compounds knowing that we would have some interesting analogues of the natural pheromone that would enable us to begin to understand structure–activity relationships. One diastereomer of structure **a** (Fig. 3) matched the natural compound in all physical, chromatographic, and spectrometric properties. The pheromone contains two chiral carbons whose absolute configurations must still be determined. The other analogues of supellapyrone have obviously different properties. Kovat's retention indices were as follows: (*i*) Carbowax: natural, 2504; synthetic diastereomers for **a**, 2504/2515; **b**, 2585/2592; **c**, 2552/2588; and **d**, 2498. (*ii*) SE-30: natural, 1806; synthetic diastereomers for **a**, 1806/1815; **b**, 1845/1851; **c**, 1836/1845; and **d**, 1809.

The pheromonal activity of the synthetic enantiomeric mix of supellapyrone was demonstrated by the two-choice olfactometer and EAG bioassays. In olfactometer assays, males responded similarly to a dose series (10^{-8}–10^{-3} ng) of the purified natural and synthetic pheromone (Fig. 4*A*). Moreover, males tested during the dark responded to extremely small quantities of pheromone. The synthetic pheromone also gave EAG responses comparable to its natural counterpart over a wide range of doses (Fig. 4*B*). Based on chromatographic data, the natural pheromone consists of, at most, two enantiomers (see Kovat's indices above). We have not determined whether the other enantiomers are biologically active. Our bioassay results did suggest, however, that the other enantiomers neither appreciably synergize nor strongly inhibit male response, as has been noted in other insects (25).

DISCUSSION

The brownbanded cockroach pheromone represents an additional class of natural products; to our knowledge it is the only 5-alkyl-3-methyl-α-pyrone so far discovered. Even the analogue, 3,5-dimethyl-α-pyrone, that we and others (18) have synthesized is not known to occur naturally. α-Pyrones having alkyl side chains in the 4 and/or 6 position are the only

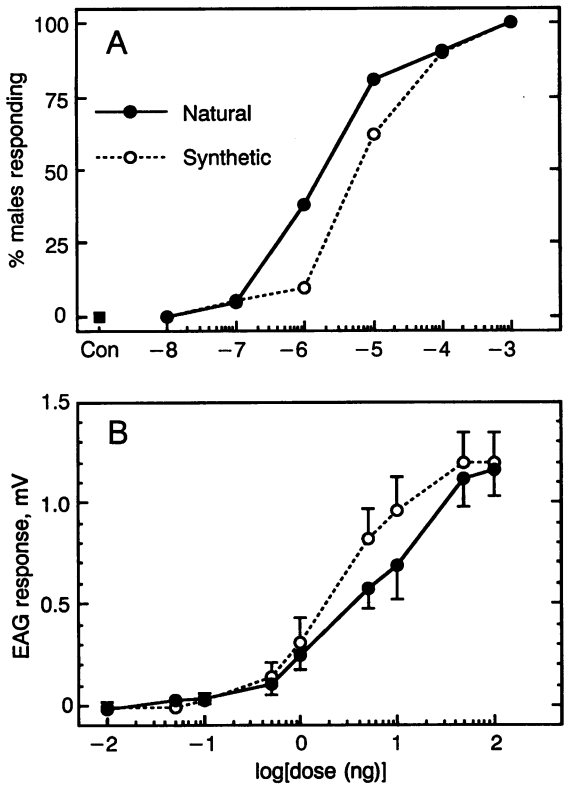

FIG. 4. (*A*) Behavioral responses of male *S. longipalpa* in the two-choice olfactometer. $n = 20$ or 21 for each point. Con, control (*B*) EAG responses of *S. longipalpa* males. $n = 4$ antennae for each mean. Control values were subtracted from each determination. Vertical bars indicate standard errors. For these experiments, we assumed that only one enantiomer was biologically active and that the synthetic pheromone contained equal amounts of the four enantiomers. Thus, to hold the amount of the active component constant, filter paper dispensers used in bioassays were loaded with a 4-fold higher amount of the synthetic than the natural material at each dose.

compounds of this class normally encountered and for which synthetic routes are available (26, 27). The latter compounds have strong organoleptic properties and thus are important to food and fragrance industries.

Pyrones have rarely been found in insects. The only other known α-pyrone is the queen recognition pheromone of the red imported fire ant (28); this same 6-alkyl-substituted compound was reported as a volatile metabolite from a soil fungus, *Trichoderma viride* (29). A few other types of pyrones do occur in insects. For example, 6-*n*-pentyl-5,6-dihydro-2-pyrone evidently is used as a defensive secretion by two species of formicine ants (30). Male ghost moths release copious amounts of their sex pheromone, alkyl-2,3-dihydro-4*H*-pyran-4-one, to attract females (31, 32). Another γ-pyrone derivative is used as a sex pheromone by two anobiid beetles (33, 34).

Because insufficient purified material was available, we were not able to determine the stereochemistry of the natural pheromone. Nevertheless, synthetic racemic supellapyrone is a powerful long-range attractant for male brownbanded cockroaches. The volatile pheromone thus should prove valuable as a trap bait to monitor cockroach populations or, when used in conjunction with an insecticide, to abet direct control efforts.

We thank D. Kiemle for obtaining NMR spectra; Bruker, Inc. (Bellerica, MA) for donating use of their 600-MHz NMR spectrometer; T. G. Hartman, J. Lech, and R. T. Rosen (Center for Advanced Food Technology, Rutgers University) for providing the chemical ionization-MS spectrum; H. Xu and K. Poole for maintaining the insects; and G. Rule for technical help. We are grateful to R. Heath and R. Murphy (Agricultural Research Station–U.S. Department of Agriculture, Gainesville, FL) for providing the FTIR spectrum and other assistance during the course of this study. This work was supported in part by a U.S. Department of Agriculture Northeast Integrated Pest Management grant.

1. Schal, C. & Smith, A. F. (1990) in *Cockroaches as Models for Neurobiology: Applications in Biomedical Research*, eds. Huber, I., Rao, B. R. & Maisler, E. P. (CRC, Boca Raton, FL), pp. 179–200.
2. Nishida, R. & Fukumi, H. (1983) *Mem. Coll. Agric. Kyoto Univ. Pesticide Sci. Ser.* **122**, 1–24.
3. Schal, C., Burns, E. L., Jurenka, R. A. & Blomquist, G. J. (1990) *J. Chem. Ecol.* **16**, 1997–2008.
4. Sreng, L. (1990) *J. Chem. Ecol.* **16**, 2899–2912.
5. Persoons, C. J., Verwiel, P. E. J., Talman, E. & Ritter, F. J. (1979) *J. Chem. Ecol.* **5**, 221–236.
6. Hauptman, H., Mühlbauer, G. & Sass, H. (1986) *Tetrahedron Lett.* **27**, 6189–6192.
7. Okada, K., Mori, M., Shimazaki, K. & Chuman, T. (1991) *J. Chem. Ecol.* **17**, 695–706.
8. Takegawa, H. & Takahashi, S. (1989) *Appl. Entomol. Zool.* **24**, 435–440.
9. Wright, C. G. (1977) *J. Georgia Entomol. Soc.* **12**, 281–283.
10. Hales, R. A. & Breed, M. D. (1983) *Ann. Entomol. Soc. Am.* **76**, 239–241.
11. Smith, A. F. & Schal, C. (1990) *J. Insect Physiol.* **36**, 251–257.
12. Liang, D. & Schal, C. (1990) *J. Insect Behav.* **3**, 211–223.
13. Schal, C., Liang, D., Hazarika, L. K., Charlton, R. E. & Roelofs, W. L. (1992) *Ann. Entomol. Soc. Am.* **85**, 605–611.
14. Heath, R. R. & Tumlinson, J. H. (1984) in *Techniques in Pheromone Research*, eds. Hummel, H. E. & Miller, T. A. (Springer, New York), pp. 287–322.
15. Kovats, E. (1965) *Adv. Chromatogr.* **1**, 229–247.
16. Liang, D. & Schal, C. (1990) *Physiol. Entomol.* **15**, 355–361.
17. Roelofs, W. (1984) in *Techniques in Pheromone Research*, eds. Hummel, H. E. & Miller, T. A. (Springer, New York), pp. 131–159.
18. Barltrop, J. A., Barrett, J. C., Carder, R. W., Day, A. C., Harding, J. R., Long, W. E. & Samuel, C. J. (1979) *J. Am. Chem. Soc.* **101**, 7510–7521.
19. Benn, R. & Gunther, H. (1983) *Angew. Chem. Int. Ed. Engl.* **22**, 350–380.
20. Bax, A. & Davis, D. G. (1985) *J. Magn. Reson.* **63**, 207–213.
21. Brownlee, R. G. & Silverstein, R. M. (1968) *Anal. Chem.* **40**, 2077–2079.
22. Johnson, W. S., Werthemann, L., Bartlett, W. R., Brockson, T. J., Li, T., Faulkner, D. J. & Peterson, M. R. (1970) *J. Am. Chem. Soc.* **92**, 741–743.
23. Malik, F., Hassan, M., Rosenbaum, D. & Duddeck, H. (1989) *Magn. Reson. Chem.* **27**, 391–395.
24. Kessler, H., Griesinger, C., Zarbock, J. H. & Looslie, R. (1984) *J. Magn. Reson.* **57**, 331–336.
25. Mori, K. (1984) in *Techniques in Pheromone Research*, eds. Hummel, H. E. & Miller, T. A. (Springer, New York), pp. 323–370.
26. Nobuhara, A. (1968) *Agric. Biol. Chem.* **32**, 1016–1020.
27. Pittet, A. O. & Klaiber, E. M. (1975) *J. Agric. Food Chem.* **23**, 1189–1195.
28. Rocca, J. R., Tumlinson, J. H., Glancey, B. M. & Lofgren, C. S. (1983) *Tetrahedron Lett.* **24**, 1889–1892.
29. Moss, M. O., Jackson, R. M. & Rogers, D. (1975) *Phytochemistry* **14**, 2706–2708.
30. Cavill, G. W. K., Clark, D. V. & Whitfield, F. B. (1968) *Aust. J. Chem.* **21**, 2819–2823.
31. Kubo, I., Matsumoto, T., Wagner, D. L. & Schoolery, J. N. (1985) *Tetrahedron Lett.* **26**, 563–566.
32. Francke, W., Mackenroth, W., Schroder, W., Schulz, S., Engels, W. & Schneider, D. (1985) *Z. Naturforsch. C* **40**, 145–147.
33. Kuwahara, Y., Fukami, H., Howard, R., Ishii, S., Matsumura, F. & Burkholder, W. E. (1978) *Tetrahedron* **34**, 1769–1774.
34. White, P. R. & Birch, M. C. (1987) *J. Chem. Ecol.* **13**, 1695–1706.