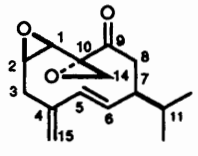
X-Ray Crystallographic and NOE Studies on the Conformation of Periplanones and their Analogues

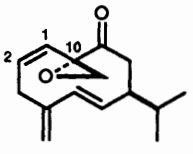
Masataka Mori,* Kentaro Okada, Kazuko Shimazaki, and Tatsuji Chuman
Life Science Research Laboratory, Japan Tobacco Inc., 6-2 Umegaoka, Midori-ku, Yokohama 227, Japan
Shigefumi Kuwahara, Takeshi Kitahara, and Kenji Mori
Department of Agricultural Chemistry, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

The structure of periplanone-A was established by X-ray crystallographic analysis. In comparison of the X-ray and ^{1}H NMR data with those of periplanone-B, it was shown that periphanone-A and -B adopt essentially the same conformation. The epoxy epimer of periplanone-A has 10^4-times lower biological activity than periplanone-A, and NMR analysis indicated that the molecule exists in a mixture of different conformers. The conformation of the regioisomer, in which the carbonyl and epoxy groups are transposed, was analysed by X-ray crystallography and ^{1}H NMR spectroscopy. It is highly biologically active, and the conformation of the germacranoid skeleton was shown to be almost identical with that of periplanone-A. Simplified analogues and germacrene-D had relatively lower activity. NOE Experiments on these compounds suggested the conformational resemblance of their germacranoid skeletons with those of periplanone-A and -B.

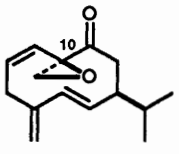
Periplanone-A (P-A) and -B (P-B) are the extremely potent sex pheromones of the American cockroach (*Periplaneta americana* L.). Since the isolation of these pheromone components by Persoons *et al.*[1]† in 1974, much attention has been focussed on the structural determination of the natural pheromones. Although the structure of P-B was established[2] as (**1**) earlier, the structure of P-A has long been a matter of discussion among several groups.[3–6] Very recently, through the chiral syntheses of the candidates and their X-ray crystallographic analyses,[7–9] the structure of P-A was shown to be (**2**), a structure proposed by Hauptmann *et al.*[4]



Periplanone-B (1)



Periplanone-A (2)



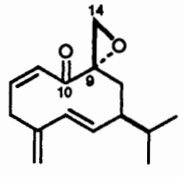
(3)

Being interested in the unique structural features and biological activity of these pheromones, we started to investigate the molecular shapes and structural requirements for the pheromonal activity. Few studies on substances pheromonally active toward the American cockroach,[10,11] however, have appeared until now, and no highly active compound with an activity threshold $>10^{-5}$ that of natural pheromones was known. Furthermore, because of the well known flexibility of 10-membered-ring systems of the germacrane skeleton,[12] it was important to consider the molecular shapes of the analogues compared with those of the leading natural pheromones.

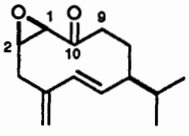
In this report, we describe the structural elucidation of P-A (**2**) and its epoxy epimer (**3**), and discuss the conformational properties and biological activity of periplanones and some germacranoid analogues‡ by means of their X-ray crystallographic data and high-field ^{1}H NMR analyses.

Results and Discussion

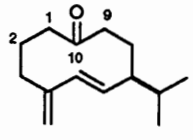
Materials.—Natural (−)-enantiomer of P-B (**1**) was a pure crystalline solid.[2] P-A (**2**)[4] and the epoxy epimer (**3**)[5] were obtained in optically active form by synthesis.[8] Since our initial interest in periplanones was the contribution of oxygen-containing functionalities on the biological activity and conformational properties, we employed the compounds (**4**)–(**7**) which were analogues altered at the epoxy and carbonyl groups of periplanones (**1**) and (**2**). Compounds (**4**)–(**6**) were obtained in chemically and optically pure forms.[2],§ The simplest molecule (**7**), (−)-germacrene-D,[10] was known as a pheromonally active natural product. All of the compounds possess a common conjugated diene and isopropyl group in their 10-membered-ring skeletons as shown by structure (**7**).



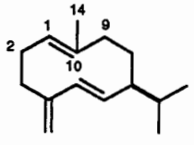
(4)



(5)



(6)



Germacrene-D (7)

Biological Tests.—For the biological evaluations of the compounds, behavioural[13] and electroantennographic (EAG)

† Recently, new pheromone components, periplanone-D_1 and -D_2, with an activity threshold of 5×10^{-2} μg were isolated and identified from the female American cockroach (M. Biendl, H. Hauptmann, and H. Sass, *Tetrahedron Lett.*, 1989, **30**, 2367).

‡ The same numbering scheme for the C atoms is employed in this paper as has been used for P-B by Persoons *et al.*[1]

§ The structure of compound (**4**) was first presented at the 16th International Symposium on the Chemistry of Natural Products, IUPAC, Kyoto, May, 1988, (Abstracts, p. 649).

assays[14] were performed. The results are summarized in Table 1. The threshold values of (1), (2), and (7) in behavioural assay were in agreement with those reported in the literatures.[15,16]

Conformational Analysis.—Since the conformational properties of compounds (3)–(7) are discussed largely on the basis of ^{1}H NMR analyses in this work, the NMR characterizations of the leading compound (1) and (2) were prerequisite for these studies. The ^{1}H NMR data for compounds (1) and (2) and the analogues (3)–(5) are listed in Tables 2 and 3, respectively.

Periplanone-B (1). The conformational properties of (1) have been described by Still in his first synthesis of the compound.[12] Recently, Hauptmann *et al.* reported the X-ray crystal structure of (1).[16] In an examination of the 500 MHz ^{1}H NMR spectrum of compound (1) in [$^2H_6$]benzene solution, the coupling-constant data provided rather poor information on the conformational properties in solution, except that the large couplings between 2-H/3-H$^\beta$ (10.2 Hz) and 7-H/8-H$^\beta$ (11.0 Hz) were due to their *anti* orientations. However, NOE enhancements were observed among a number of non-coupled protons on the molecule in the NOESY spectrum as depicted in Figure 1. The olefinic 6-H signal interacts with those of 3-H$^\beta$, 8-H$^\beta$, and 11-H, and that of 5-H interacts with that of 7-H. As a relatively large NOE enhancement (6.0%) was observed at 3-H$^\beta$ on irradiation of the 6-H signal, it reflects their close intramolecular distance (2.13 Å from X-ray data). These results for the solution conformation of P-B (1) were essentially consistent with that in the solid state.

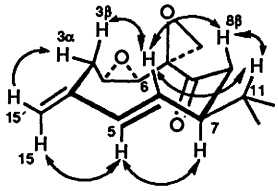


Figure 1. Stereostructure of periplanone-B (1) and intramolecular ^{1}H NOE interactions observed in C_6D_6 solution.

Table 1. Bioassay data of periplanones and the analogues.

	Behavioural test threshold (μg)	EAG response[a]
P-B (1)	10^{-7}	100
P-A (2)	10^{-5}	85
(3)	10^{-1}	32
(4)	10^{-4}	25
(5)	10^{-1}	18
(6)	1	13
(7)	10	11
Control[b]		8

[a] Relative intensities at 10^{-1} μg doses to that of P-B (1) (= 100). [b] Hexane (1 μl).

Periplanone-A (2) *and the epoxy epimer* (3). Owing to the ambiguity of the stereochemistry of the quaternary centre at the spiro-epoxy group, two structures (2)[4] and (3),[5] which are epimeric at C-10, have been proposed for P-A. To clarify this point, both of these isomers were synthesized in their optically active form,[8] and subjected to precise stereochemical analyses. The biologically active epimer, of which the NMR spectrum is identical with that reported by Hauptmann *et al.*,[4] is a crystalline compound and the X-ray analysis established the structure to be (2). Compound (2) crystallized with three molecules, the shapes of which are essentially identical, in the asymmetric crystallographic unit-cell. The ORTEP stereo projections of the three molecules and unit-cell contents are shown in Figures 2 and 3, respectively.

A computer-aided superimposition of the X-ray structures (Figure 4) showed that the molecular shape of P-A (2) (molecule A) apparently overlaps that of P-B (1) except for the absence of the *endo* epoxy group at C-1 and -2. A ^{1}H NMR inspection of compound (2) also shows similar coupling and NOE patterns with those of P-B (1) (Figure 5). Lack of NOE interactions of 6-H with the 8-H$^\beta$ and 11-H signals is a distinction from the situation with P-B (1), and indicates the differences in the solution conformation.

On the other hand, the epoxy epimer (3) exhibits 10^4-times lower biological activity than does P-A (2). In contrast to compound (2), the NOESY spectrum (Figure 6) was so complicated that we could not define the conformation of compound (3). Namely, the signal for 6-H shows cross peaks to those of 2-, 3-, 3'-, 7-, and 11-, and 5-H interacts with 7-H and 11-. This spectral feature suggested that the molecule compound (3) may exist in a mixture of conformers different from those of P-A (2).

As seen in the crystal structure of P-A (2), the epoxy oxygen atom is positioned in close contact with 3-H$^\beta$, 6-H, and 8-H$^\beta$ ring hydrogens; replacement of the oxygen with a bulky methylene group as in isomer (3) may cause a severe steric

Table 2. ^{1}H NMR data (500 MHz) of periplanone-B (1) and -A (2) in C_6D_6.

	(1)			(2)		
	δ	Mult.	*J* (Hz)	δ	Mult.	*J* (Hz)
1	3.84	d	4.0	6.03	d	11.8
2	2.74	dt	10.2, 4.0	5.53	ddd	11.8, 11.0, 7.4
3$^\beta$	2.86	dd	12.2, 10.2	3.95	br t	11.0
3$^\alpha$	2.56	dd	12.2, 4.0	2.45	dd	11.0, 7.4
5	5.96	d	16.0	6.00	d	16.2
6	5.91	dd	16.0, 9.0	6.18	dd	16.2, 11.0
7	2.05	m		2.11	m	
8$^\beta$	2.35	dd	11.0, 9.8	2.44	dd	11.8, 9.6
8$^\alpha$	1.94	dd	9.8, 5.7	1.93	dd	9.6, 5.2
11	1.30	m		1.36	m	
12	0.72	d	6.9	0.77	d	7.4
13	0.70	d	6.8	0.73	d	6.6
14	2.63	d	5.7	2.30	d	5.6
14'	2.02	d	5.7	2.16	d	5.6
15	4.81	br s		4.84	br s	
15'	4.78	br s		4.75	br s	

Table 3. ^{1}H NMR data (500 MHz) of periplanone analogues (**3**), (**4**), and (**5**) in C_6D_6.

	(3)			(4)			(5)		
	δ	Mult.	*J* (Hz)	δ	Mult.	*J* (Hz)	δ	Mult.	*J* (Hz)
1	5.95	d	11.2	5.82	d	12.0	3.03	d	4.7
2	5.44	dt	11.2, 8.6	5.54	ddd	12,0, 10,3, 6.6	2.79	ddd	9.9, 4.7, 3.5
3$^\alpha$	3.16[a]	br dd	12.2, 8.6	3.77	dd	12.0, 10.3	2.52	dd	12.7, 3.5
3$^\beta$	2.66[a]	dd	12.2, 8.6	2.48	dd	12.0, 6.6	2.35	dd	12.7, 9.9
5	5.91	d	16.5	6.04	d	16.2	5.66	d	16.2
6	5.59	dd	16.5, 8.3	5.43	dd	16.2, 10.5	5.10	dd	16.2, 10.3
7	2.06	m		2.11	m		1.29	m	
8$^\alpha$	2.35[a]	dd	10.0, 5.1	2.82	dd	13.6, 11.9	2.09	m	
8$^\beta$	2.09[a]	dd	10.0, 7.4	1.15	dd	13.6, 4.8	1.94	br q	−12
9							1.85	ddd	16.1, 12.5, 1.1
9′							1.64	ddd	16.1, 6.5, 1.2
11	1.50	m		1.35	m		1.29	m	
12	0.81	d	6.6	0.79	d	7.3	0.78	d	6.2
13	0.77	d	6.7	0.77	d	6.5	0.75	d	6.2
14	2.61	d	5.8	2.27	d	4.7			
14′	2.40	d	5.8	2.17	d	4.7			
15	4.80	br s		4.81	br s		4.76	br s	
15′	4.76	br s		4.72	br s		4.74	br s	

[a] Configurations were not assignable.

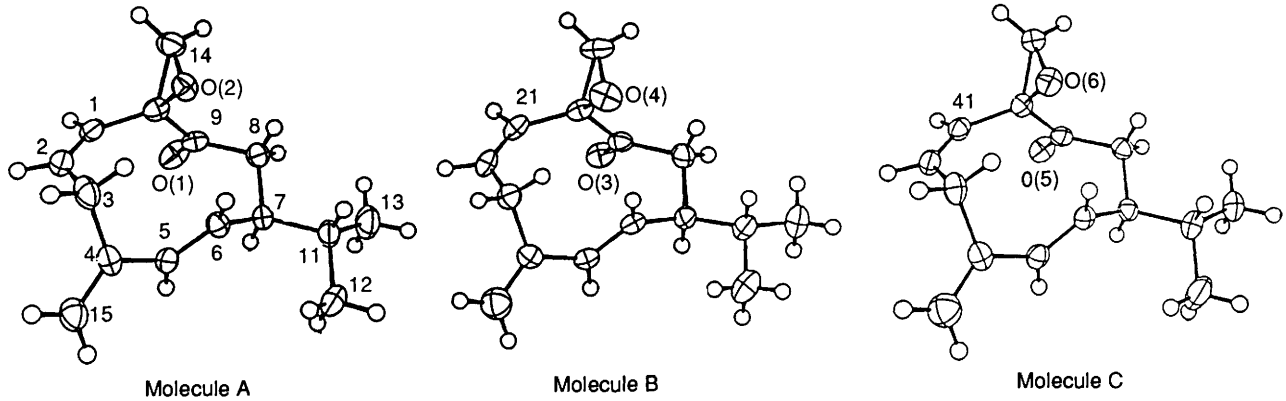


Figure 2. ORTEP drawing of the three independent molecules of periplanone-A (**2**).

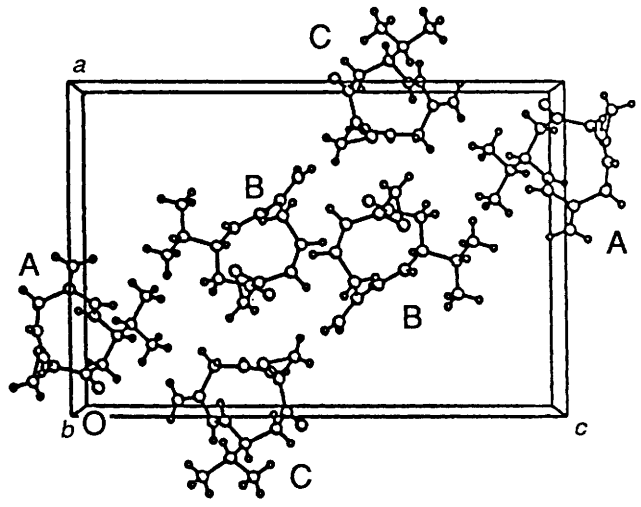


Figure 3. Crystal-packing diagram of periplanone-A (**2**) viewed along the *b* axis.

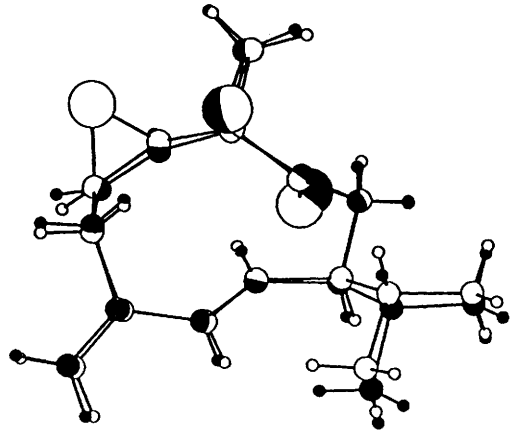


Figure 4. Superimposition of the crystal structures of periplanone-A (**2**) (filled) and -B (**1**). Oxygen atoms are shown by large circles.

repulsion. Furthermore, the chiroptical properties[8] of compound (**3**) (+182°) were quite different from those of P-A (**2**) (−574°). Therefore, we suppose that compound (**3**) could not exist in a conformation like that of compound (**2**).

Compound (**4**). Compound (**4**)* was prepared in the course

* The structure of compound (**4**) was first presented at the 16th International Symposium on the Chemistry of Natural Products, IUPAC, Kyoto, May 1988, (Abstracts, p. 649).

of our synthetic study[2] of (−)-periplanone-B (**1**). When the synthetic intermediate (**8**) was treated with the sulphonium ylide prepared from trimethylsulphonium iodide and dimsyl sodium [$MeS(O)CH_2^-Na^+$], a remarkable side-reaction occurred to give some abnormal products. Use of butyl-lithium as base gave an expected bisepoxide (**9**) in good yield (Scheme). From the former reaction mixture, we isolated two products, both of which exhibited significant biological activity. Surprisingly, the minor product was spectroscopically identical with periplanone-A (**2**). The structure of the major product, the biological activity of which is one order of magnitude lower than that of P-A (**2**), was established by X-ray analysis to be (**4**), a regioisomer of P-A (**2**) (Figure 7).

As seen in Figure 8, the skeletal conformation of compound (**4**) overlaps well with that of P-A (**2**). An NOE experiment indicated the conformation of compound (**4**) in solution (Figure 9). It is noteworthy that a transposition of carbonyl and spiro-epoxy group did not affect the placements of each oxygen atom in the molecules.

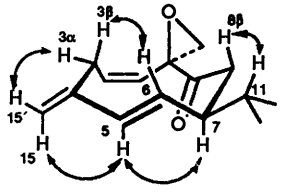


Figure 5. Stereostructure of periplanone-A (**2**) and intramolecular ^1H NOE interactions observed in C_6D_6 solution.

Compound (**5**). Compound (**5**)[2,17] is a simplified analogue of P-B (**1**), which lacks the carbonyl group at C-9 and possesses a carbonyl group instead of the spiro-epoxy group at C-10, but still generates activity (threshold 10^{-1} μg). In contrast to the above mentioned compounds, much information concerning the conformation of this compound was obtained in the NMR analysis. The assigned alignments of spin-couplings along 2-H to 3-H, and 7-H to 9-H, and the results of NOE experiments (Figure 10) clearly showed that compound (**5**) lay in a similar conformation to P-B (**1**) in solution. As an NOE interaction was observed between 1-H and 9-H$^{\alpha}$, the carbonyl function at C-10 may be vertical with respect to the ring plane.

Compound (**6**)[2,17] *and Germacrene-D* (**7**).[10] Each of the more simplified analogues exhibited a lower activity than did compound (**5**). On the elucidation of the ^1H NMR spectra of compounds (**6**) and (**7**), the multiplicity of signals for the ring protons is complicated by considerable overlapping and AB-fusions [NMR data for compounds (**6**) and (**7**) are given in Experimental section]. However, in the spectrum of compound (**6**), the signals of the geminal methylenes at C-3 and -8 are well resolved from other signals. From an analogy with the spectral features of compound (**5**), one of the 3-H signals at δ 2.55, which has a large coupling constant with 2-H, was tentatively assigned

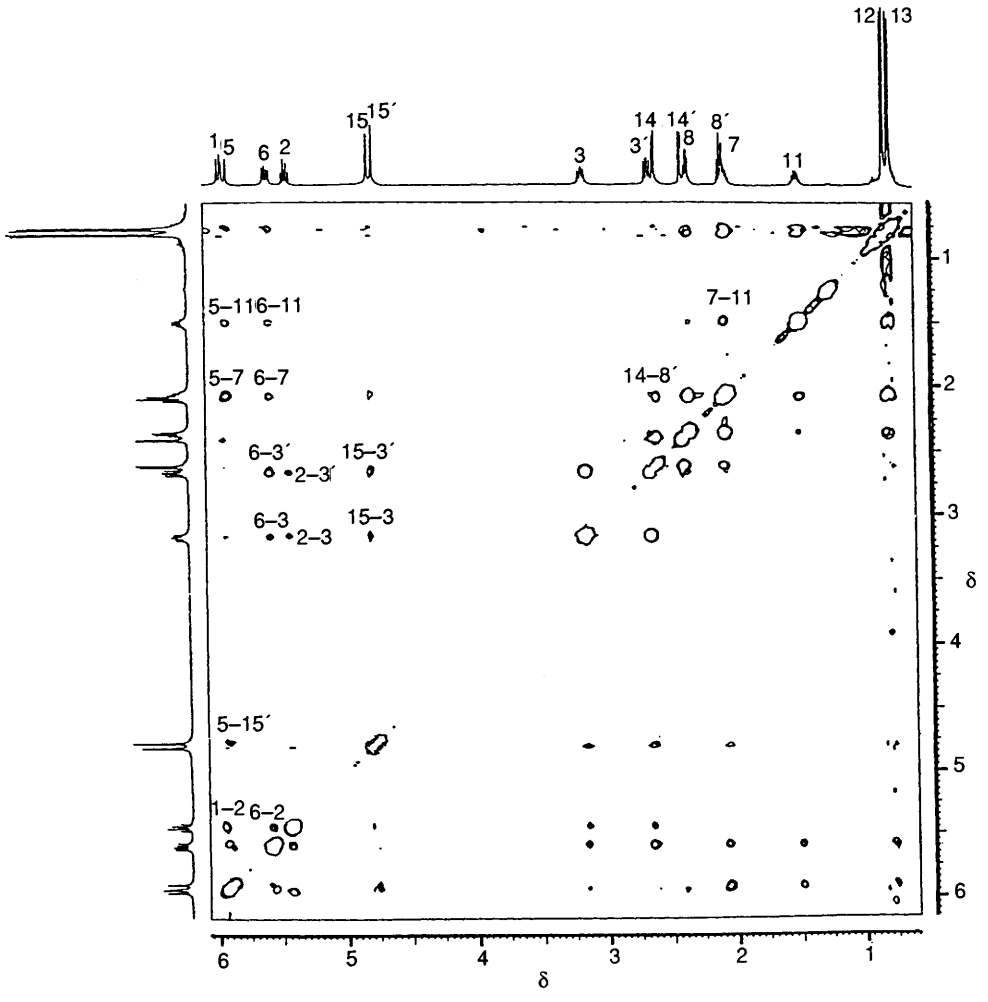
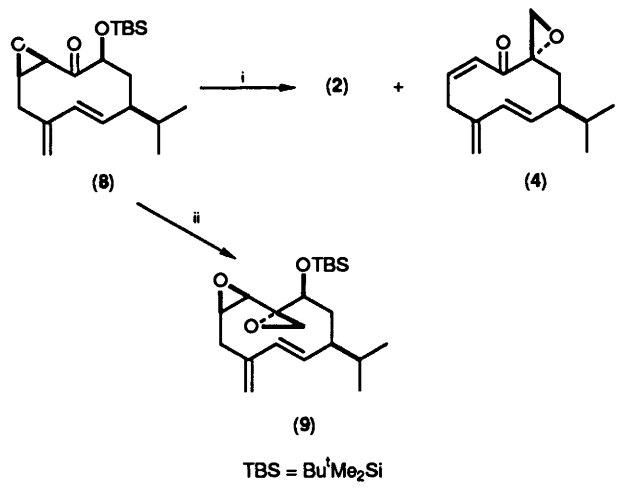


Figure 6. Phase-sensitive 2D NOESY spectrum of compound (**3**) recorded at 500 MHz in C_6D_6 solution, and partial assignments of the cross-peaks; dispersed cross-peaks between 5-H and 6-H are due to their direct coupling.



TBS = Bu^tMe_2Si

Scheme. *Reagents:* i, Me_3SI, $MeS(O)CH_2^-Na^+$, Me_2SO–THF; ii, Me_3SI, BuLi, THF.

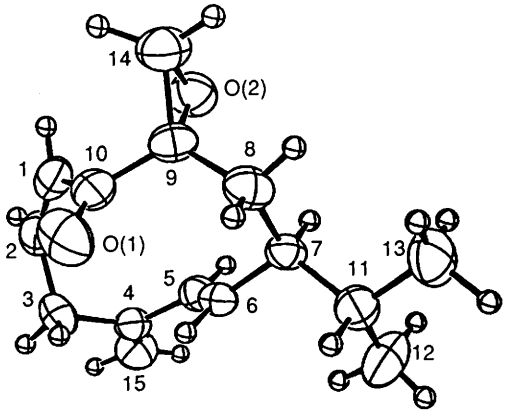


Figure 7. ORTEP drawing of compound (**4**).

to that of a β-orientation proton. The signal at δ 2.09 was likewise assignable to 8-H^{β}. On irradiation of the signal due to 6-H, a significant NOE enhancement (5.8%) was observed for 3-H^{β}, along with weak interactions at the 8-H^{β} (1.4%) and 11-H (1.7%) signals (Figure 10). These spectral features are characteristic of a conformation like that of compounds (**1**) and (**5**).

Similarly, no coupling data for sp^3 protons of compound (**7**) were obtained because of the more complex multiplicity of the signals. However, NOE interactions were observed among some signals of olefinic and methyl protons of compound (**7**), as depicted in Figure 11. A transannular NOE interaction between the methyl at C-10 and the 6-H proton indicated that the methyl group may be located on the β side of the ring. This evidence suggested that the molecule (**7**) may also adopt a similar conformation to that of the other compounds examined in this study.

Conclusions

On analysis of the biological activity and structural features of the periplanones and their analogues, it was seen that the conformational analogy of the germacranoid skeleton and the oxygen-containing functionalities must play an important role in the exhibition of biological activity. The characteristic three-dimensional locations of the oxygen atoms in the carbonyl and spiro-epoxy groups on the ten-membered-ring planes may also be necessary in order to exhibit higher activity. As seen in the case of compound (**3**), stereochemical restrictions are essential in the conformational properties of the ten-membered ring. These findings on biological activity and structural factors could provide a guideline towards designing useful analogues for the control of the cockroach insect pest. For molecular recognition not only the size but also the shape of the electropotential surface of the molecule is critical. We think that the conformational assignments of the compounds described in this study are valuable for possible future studies of the electronic aspects of periplanones.

Recent electrophysiological studies[18] suggested the presence of two distinct receptor cells for P-A and P-B on an antenna of the American cockroach. Such a high specificity which can strictly distinguish these closely analogous molecular shapes may be a striking character of the receptor cells.

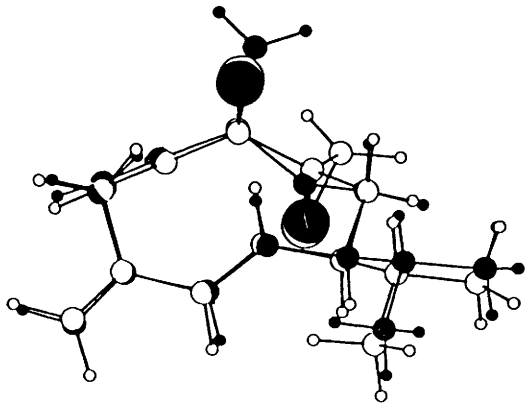


Figure 8. Superimposition of the crystal structures of periplanone-A (**2**) (filled) and compound (**4**). Oxygen atoms are shown by large circles.

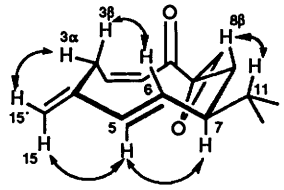


Figure 9. Stereostructure of compound (**4**) and intramolecular ^1H NOE interactions observed in C_6D_6 solution.

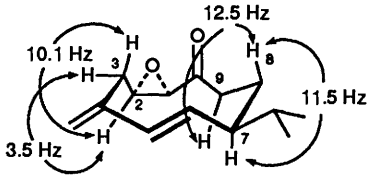
Experimental

General Methods.—M.p.s were measured on a Yanagimoto micro-melting-point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT/IR-5000 spectrometer. GLC-MS spectra (70 eV) were run on a JEOL DX-300 spectrometer. ^1H NMR (500 MHz) spectra were recorded at 27 °C on a Bruker AM-500 instrument, and were measured in C_6D_6 solutions with Me_4Si as internal standard unless otherwise stated. $CDCl_3$ was not used so as to avoid decomposition of the acid-sensitive substrates during measurement of two-dimensional (2D) spectra. For the 2D-NOESY and NOE difference spectra (NOEMULT),[19] Bruker standard software was employed. The NOESY spectra were obtained in the phase-sensitive mode with 1K × 1K data points. PLC separations were performed on JASCO 880-PU system equipped with a JASCO 875-UV detector. Computer-aided superimpositions of X-ray crystallographic data were performed using the QUANTA™ program.* Detailed procedures of the biological test and electroantennographic assay were described in the literature.[13,14]

* Copyright Polygen Corporation, 1987, USA.

Table 4. Crystallographic data for P-A (2) and compound (4).

	(2)	(4)
Empirical formula	$C_{15}H_{20}O_2$	$C_{15}H_{20}O_2$
Formula weight	232.32	232.32
Crystal system	monoclinic	orthorhombic
Space group	$P2_1$	$P2_12_12_1$
Cell dimensions		
a (Å)	14.650(2)	12.3186(8)
b (Å)	6.469(2)	16.044(1)
c (Å)	21.361(1)	6.8292(5)
β	90.702(8)°	
Cell volume (Å^3)	2 024.5(6)	1 349.7(2)
Z	6	4
D_{calc} (g cm^{-3})	1.143	1.14
Number of reflections		
Measured	3 459 (3 313 unique)	1 149
Observed [$I > 3\sigma(I)$]	3 005	897
R, R_W	0.038, 0.047	0.034, 0.041



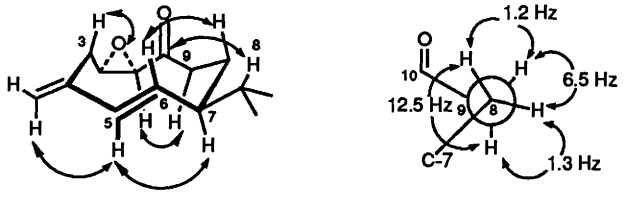


Figure 10. Stereostructure of compound (5) and its ^{1}H NMR spectral properties (right: coupling constants; left: NOE interactions).

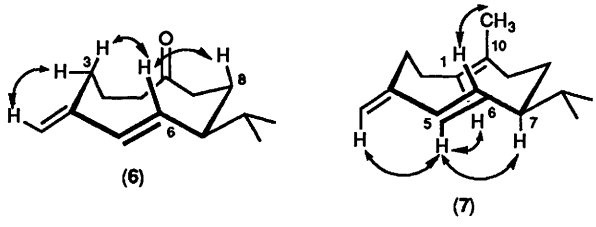


Figure 11. Proposed stereostructures of compound (6) and germacrene-D (7), and their NOE interactions.

Materials.—Optically pure crystalline (−)-P-B (1) (99.95% e.e.) was obtained by our previous procedure.[2] A mixture of P-A (2) and the epoxy epimer (3) was synthesized from (*R*)-(+)-cyclohex-3-enecarboxylic acid ($[\alpha]_D^{17}$ +98°) by the procedure reported by Kuwahara and Mori,[8] and were separated on preparative TLC (PLC) (twice). Three recrystallizations gave pure crystalline compound (2). The oily compound (3) was further purified on HPLC [column, YMC A-024 SIL (10 × 300 mm); solvent hexane–ethyl acetate (98:2), flow rate 4 ml/min; t_R 25.2 min for (2), 38.2 min for (3)].

Compounds (5) and (6) were the synthetic intermediates in the synthesis[2] of (−)-P-B (1). Compounds (5) and (6) have also been described by Schreiber and Santini[17] in their synthesis of (±)-(1). Each of these two samples was chromatographically pure and the optical purity was ≥98% e.e. However, capillary GLC-MS analysis of compound (5) showed contamination of a stereoisomer at the epoxy group. A pure crystalline sample, m.p. 52–53 °C, $[\alpha]_D^{25}$ −378° (*c* 0.533, hexane), was obtained on HPLC purification [column, YMC A-012 SIL; solvent hexane–ethyl acetate (96:4), flow rate 1.0 ml/min; t_R 27.4 min for (5); t_R 32.3 min for the isomer of (5)].

Germacrene-D (7), isolated from ylang-ylang essential oil, was a gift from Dr. Y. Takagi of T. Hasegawa Inc.; the chemical purity was 98% as judged by GLC.

The Formation of P-A (2) *and of Compound* (4).—A solution of sodium methylsulphinyl carbanion (1.0M; 0.1 ml) in dry di-

Table 5. Non-hydrogen atom co-ordinates for compound (2) (e.s.d.s in parentheses).

Atom	*x*	*y*	*z*
	Molecule (A)		
O(1)	0.936 7(2)	0.7917	0.996 0(1)
O(2)	0.832 0(2)	0.540 5(6)	1.098 3(1)
C(1)	0.842 0(2)	0.920 3(7)	1.075 5(1)
C(2)	0.757 9(2)	0.981 4(7)	1.086 8(1)
C(3)	0.672 3(2)	0.853 8(7)	1.086 4(1)
C(4)	0.627 2(2)	0.866 3(7)	1.021 6(1)
C(5)	0.675 3(2)	0.752 9(7)	0.972 0(1)
C(6)	0.714 6(2)	0.568 9(7)	0.978 4(1)
C(7)	0.778 9(2)	0.471 8(7)	0.933 1(1)
C(8)	0.872 8(2)	0.453 9(7)	0.966 8(1)
C(9)	0.898 3(2)	0.659 5(7)	0.995 4(1)
C(10)	0.873 7(2)	0.705 1(7)	1.062 8(1)
C(11)	0.745 3(2)	0.261 0(7)	0.908 2(1)
C(12)	0.654 1(2)	0.289 3(8)	0.873 9(2)
C(13)	0.813 7(3)	0.156 8(8)	0.865 6(2)
C(14)	0.925 7(2)	0.588 7(8)	1.110 9(1)
C(15)	0.556 3(2)	0.986 3(8)	1.009 8(2)
	Molecule (B)		
O(3)	0.642 3(1)	0.925 3(6)	0.603 5(1)
O(4)	0.568 5(1)	1.411 7(6)	0.668 4(1)
C(21)	0.571 4(2)	1.302 2(7)	0.555 6(1)
C(22)	0.488 0(2)	1.343 1(7)	0.634 4(1)
C(23)	0.401 4(2)	1.369 4(7)	0.570 9(1)
C(24)	0.352 2(2)	1.163 9(7)	0.576 3(1)
C(25)	0.392 7(2)	1.013 7(7)	0.620 1(1)
C(26)	0.435 9(2)	1.060 7(7)	0.673 7(1)
C(27)	0.492 5(2)	0.916 6(7)	0.712 0(1)
C(28)	0.593 7(2)	0.989 6(7)	0.708 0(1)
C(29)	0.615 3(2)	1.050 2(7)	0.641 8(1)
C(30)	0.602 5(2)	1.273 9(7)	0.621 5(1)
C(31)	0.462 1(2)	0.902 9(8)	0.780 5(1)
C(32)	0.520 9(3)	0.752(1)	0.818 1(2)
C(33)	0.362 5(3)	0.841(1)	0.784 3(2)
C(34)	0.662 9(2)	1.427 8(7)	0.652 0(2)
C(35)	0.282 3(2)	1.115 9(7)	0.539 7(2)
	Molecule (C)		
O(5)	−0.044 5(1)	0.441 3(6)	0.460 8(1)
O(6)	0.147 1(1)	0.677 0(5)	0.383 7(1)
C(41)	0.113 3(2)	0.298 5(7)	0.396 9(1)
C(42)	0.129 4(2)	0.225 9(7)	0.339 9(1)
C(43)	0.132 8(2)	0.343 1(7)	0.279 0(1)
C(44)	0.039 3(2)	0.327 7(7)	0.246 8(1)
C(45)	−0.034 6(2)	0.446 7(7)	0.276 4(1)
C(46)	−0.024 9(2)	0.629 7(7)	0.303 6(1)
C(47)	−0.091 6(2)	0.736 3(7)	0.345 4(1)
C(48)	−0.043 9(2)	0.767 9(7)	0.409 8(1)
C(49)	−0.001 8(2)	0.567 2(7)	0.431 1(1)
C(50)	0.096 6(2)	0.516 9(7)	0.415 6(1)
C(51)	−0.127 5(2)	0.941 1(7)	0.317 7(1)
C(52)	−0.187 1(3)	0.895 6(9)	0.260 1(2)
C(53)	−0.179 6(2)	1.071 8(7)	0.364 1(2)
C(54)	0.167 5(2)	0.638 2(7)	0.449 0(1)
C(55)	0.024 7(2)	0.197 9(7)	0.200 1(2)

Table 6. Bond lengths (Å) involving the non-hydrogen atoms for compound (**2**) (e.s.d.s in parentheses).

Bond	Distance	Bond	Distance
O(1)–C(9)	1.205(4)	C(26)–C(27)	1.486(5)
O(2)–C(10)	1.437(4)	C(27)–C(28)	1.559(4)
O(2)–C(14)	1.435(4)	C(27)–C(31)	1.538(4)
C(1)–C(2)	1.319(5)	C(28)–C(29)	1.506(4)
C(2)–C(3)	1.501(5)	C(29)–C(30)	1.522(5)
C(3)–C(4)	1.529(4)	C(30)–C(21)	1.485(4)
C(4)–C(5)	1.475(4)	C(30)–C(34)	1.479(5)
C(4)–C(15)	1.319(5)	C(31)–C(32)	1.527(6)
C(5)–C(6)	1.329(5)	C(31)–C(33)	1.517(5)
C(6)–C(7)	1.497(4)	O(5)–C(49)	1.211(4)
C(7)–C(8)	1.550(4)	O(6)–C(50)	1.435(4)
C(7)–C(11)	1.541(5)	O(6)–C(54)	1.445(4)
C(8)–C(9)	1.509(5)	C(41)–C(42)	1.329(4)
C(9)–C(10)	1.516(4)	C(42)–C(43)	1.506(4)
C(10)–C(1)	1.494(5)	C(43)–C(44)	1.529(4)
C(10)–C(14)	1.479(5)	C(44)–C(45)	1.478(4)
C(11)–C(12)	1.528(5)	C(44)–C(55)	1.318(5)
C(11)–C(13)	1.520(5)	C(45)–C(46)	1.325(5)
O(3)–C(29)	1.218(4)	C(46)–C(47)	1.500(4)
O(4)–C(30)	1.435(4)	C(47)–C(48)	1.548(4)
O(4)–C(34)	1.436(4)	C(47)–C(51)	1.541(5)
C(21)–C(22)	1.325(4)	C(48)–C(49)	1.506(5)
C(22)–C(23)	1.507(4)	C(49)–C(50)	1.519(4)
C(23)–C(24)	1.517(5)	C(50)–C(41)	1.490(5)
C(24)–C(25)	1.470(5)	C(50)–C(54)	1.478(4)
C(24)–C(35)	1.318(5)	C(51)–C(52)	1.529(5)
C(25)–C(26)	1.336(4)	C(51)–C(53)	1.516(5)

Table 7. Bond angles (°) involving the non-hydrogen atoms for compound (**2**) (e.s.d.s in parentheses).

Angle		Angle	
C(10)–O(2)–C(14)	62.0(2)	C(30)–C(4)–C(34)	62.0(2)
O(2)–C(10)–C(14)	58.9(2)	O(4)–C(30)–C(34)	59.0(2)
O(2)–C(14)–C(10)	59.0(2)	O(4)–C(34)–C(30)	59.0(2)
O(1)–C(9)–C(8)	122.0(3)	O(3)–C(29)–C(28)	122.1(3)
O(1)–C(9)–C(10)	118.3(3)	O(3)–C(39)–C(30)	118.7(3)
C(2)–C(1)–C(10)	127.3(3)	C(22)–C(21)–C(30)	128.3(3)
C(1)–C(2)–C(3)	128.1(3)	C(21)–C(22)–C(23)	128.6(3)
C(2)–C(3)–C(4)	109.1(3)	C(22)–C(23)–C(24)	110.1(3)
C(3)–C(4)–C(15)	122.3(3)	C(23)–C(24)–C(35)	121.9(3)
C(5)–C(4)–C(15)	122.5(3)	C(25)–C(24)–C(35)	121.8(3)
C(3)–C(4)–C(5)	114.9(3)	C(23)–C(24)–C(25)	116.1(3)
C(4)–C(5)–C(6)	125.5(3)	C(24)–C(25)–C(26)	125.4(3)
C(5)–C(6)–C(7)	125.8(3)	C(25)–C(26)–C(27)	125.7(3)
C(6)–C(7)–C(11)	113.2(3)	C(26)–C(27)–C(31)	113.2(3)
C(8)–C(7)–C(11)	111.8(3)	C(28)–C(27)–C(31)	110.8(3)
C(6)–C(7)–C(8)	107.0(2)	C(26)–C(27)–C(28)	107.8(3)
C(7)–C(8)–C(9)	109.6(3)	C(27)–C(28)–C(29)	109.9(2)
C(8)–C(9)–C(10)	119.7(3)	C(28)–C(29)–C(30)	119.2(3)
O(2)–C(10)–C(1)	117.6(3)	O(4)–C(30)–C(21)	118.7(3)
O(2)–C(10)–C(9)	116.8(3)	C(4)–C(30)–C(29)	115.8(3)
C(1)–C(10)–C(9)	115.6(3)	C(21)–C(30)–C(29)	115.0(3)
C(1)–C(10)–C(14)	120.5(3)	C(21)–C(30)–C(34)	120.7(3)
C(9)–C(10)–C(14)	115.8(3)	C(29)–C(30)–C(34)	116.3(3)
C(7)–C(11)–C(12)	109.4(3)	C(27)–C(31)–C(32)	111.7(3)
C(7)–C(11)–C(13)	112.9(3)	C(27)–C(31)–C(33)	110.9(3)
C(12)–C(11)–C(13)	110.2(3)	C(32)–C(31)–C(33)	109.8(4)
C(50)–O(6)–C(54)	61.4(2)	C(46)–C(47)–C(51)	112.9(2)
O(6)–C(50)–C(54)	59.2(2)	C(48)–C(47)–C(51)	112.0(3)
O(6)–C(54)–C(50)	59.4(2)	C(46)–C(47)–C(48)	107.4(2)
O(5)–C(49)–C(48)	121.8(3)	C(47)–C(48)–C(49)	109.5(3)
O(5)–C(49)–C(50)	117.9(3)	C(48)–C(49)–C(50)	120.3(3)
C(42)–C(41)–C(50)	127.8(3)	O(6)–C(50)–C(41)	117.7(2)
C(41)–C(42)–C(43)	128.4(3)	O(6)–C(50)–C(49)	116.2(3)
C(42)–C(43)–C(44)	108.5(3)	C(41)–C(50)–C(49)	114.9(3)
C(43)–C(44)–C(55)	121.2(3)	C(41)–C(50)–C(54)	121.1(3)
C(45)–C(44)–C(55)	122.9(3)	C(49)–C(50)–C(54)	116.4(3)
C(43)–C(44)–C(45)	115.5(3)	C(47)–C(51)–C(52)	109.4(3)
C(44)–C(45)–C(46)	125.3(3)	C(47)–C(51)–C(53)	113.6(3)
C(45)–C(46)–C(47)	127.2(3)	C(52)–C(51)–C(53)	110.3(3)

methyl sulphoxide (DMSO) was added to a stirred suspension of trimethylsulphonium iodide (30 mg) in DMSO–tetrahydrofuran (THF) (1:1; 2 ml) at 0 °C under Ar, and the mixture was stirred for 5 min. Then a solution of compound (**8**) (17.0 mg) in dry THF (0.3 ml) was added to the mixture at −10 °C. After being stirred at between −10 and 0 °C for 15 min, the reaction mixture was quenched with saturated aq. NH_4Cl and extracted twice with hexane. The organic phase was washed successively with water and brine, and concentrated. The concentrate was chromatographed over silica gel (Mallinckrodt SilicAR CC-7™; 2 g) to remove polar products and the fractions eluted with hexane–ethyl acetate (95:5–9:1) were combined. TLC analysis showed several components along with the unchanged compound (**8**) and traces of compound (**9**). The two major components were isolated from the crude products on HPLC [column YMC A-012 SIL; solvent hexane–THF (99:1), flow rate 3.0 ml/min; t_R 3.6 min for (**2**) (1.4 mg); t_R 5.8 min for (**4**) (5.2 mg)]. Upon evaporation of the solvents, both of these two products crystallized readily, and recrystallizations from pentane–di-isopropyl ether gave pure samples.

Compound (**4**). This showed m.p. 110–110.5 °C; $[\alpha]_D^{25}$ −97.9° (*c* 0.034, hexane). The full assignment of 500 MHz ^{1}H NMR data in [$^2H_6$]benzene solution are listed in Table 3; ν_{max}(KBr, film) 3 070w, 2 975m, 2 920m, 2 869m, 1 679s, 1 653m, 1 622s, 1 612s, 1 460m, 1 391m, 1 082m, 982s, 920s, 905s, 781s, and 761s cm^{-1}; *m/z* 232 (M^+, 0.5%), 203 (0.8), 201 (1.4), 189 (7), 171 (11), 159 (16), 143 (31), 131 (32), 105 (74), and 91 (100).

^{1}H *NMR Data for Compound* (**6**) *and Germacrene-D* (**7**).—Compound (**6**) δ 2.28 (2 H, AB-m, 1-H), 1.86 (1 H, m*, 2-H), 1.20 (1 H, m, 2-H′), 2.55 (1 H, dt, *J* 4.6, ~12.6 Hz, 3-H), 2.00 (1 H, ddd, *J* 12.6, 5.7, 1.8 Hz, 3-H′), 5.90 (1 H, d, *J* 15.9 Hz, 5-H), 5.24 (1 H, dd, *J* 15.9, 10.4 Hz, 6-H), 1.46 (1 H, m, 7-H), 2.09 (1 H, m, 8-H), 1.57 (1 H, m, 8-H′), 1.91 (1 H, m*, 9-H), 1.88 (1 H, m*, 9-H′), 1.32 (1 H, m, 11-H), 0.80 (3 H, d, *J* 6.7 Hz, 12-H_3), 0.78 (3 H, d, *J* 6.7 Hz, 13-H_3), 4.85 (1 H, br s, 15-H), and 4.82 (1 H, br s, 15-H′) (* overlapped signals).

Compound (**7**). δ 5.17 (1 H, m, 1-H), ~2.3 (2 H, m, 2-H_2), ~2.0 (2 H, m, 3-H_2), 5.75 (1 H, d, *J* 15.8 Hz, 5-H), 5.25 (1 H, dd, *J* 15.8, 9.9 Hz, 6-H), ~1.9 (1 H, m, 7-H), ~1.25 (2 H, m, 8-H_2), ~2.2 (2 H, m, 9-H_2), ~1.3 (1 H, m, 11-H), 0.88 (3 H, d, *J* 6.7 Hz, 12-H_3), 0.84 (3 H, d, *J* 6.8 Hz, 13-H_3), 1.45 (3 H, br s, 14-H_3), 4.93 (1 H, br s, 15-H), and 4.79 (1 H, br s, 15-H′).

X-Ray Crystal Structure Analysis of Compound (**2**).—A colourless, needle-shaped crystal of compound (**2**) having dimensions of 0.60 × 0.12 × 0.05 mm was mounted on a glass fibre at a temperature of −120 ± 1 °C. An experiment at room temperature resulted in sublimation of the crystal. Data collection was made on a Rigaku AFC5R diffractometer with graphite-monochromated Cu-K_α radiation and a 12 kW rotating anode generator. The crystallographic data for compound (**2**) are summarized in Table 4. The structure of compound (**2**) was solved by direct methods using SHELX-86[20] and DIRDIF[21] programs. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in the structure factor calculations in idealized positions (d_{C-H} 0.95 Å), and were assigned isotopic thermal parameters which were 20% greater than the B_{equiv}-value of the atom to which they were bonded. The final cycle of full-matrix least-squares refinement converged with unweighted and weighted agreement factors of $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_W = \{[\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2]\}^{\frac{1}{2}}$. The standard deviation of unit weight was 1.48. The weighting scheme was based on counting statistics and included a factor

Table 8. Non-hydrogen atom co-ordinates for compound (4) (e.s.d.s in parentheses).

Atom	*x*	*y*	*z*
O(1)	0.721 0(2)	0.595 0(2)	0.754 4(5)
O(2)	0.500 9(2)	0.474 1(1)	0.526 1(4)
C(1)	0.643 8(2)	0.587 1(2)	0.417 8(6)
C(2)	0.642 4(3)	0.663 6(2)	0.347 5(7)
C(3)	0.439 6(3)	0.741 1(2)	0.462 7(7)
C(4)	0.518 5(3)	0.757 7(2)	0.463 7(5)
C(5)	0.452 9(3)	0.697 6(2)	0.575 7(5)
C(6)	0.476 2(3)	0.668 0(2)	0.751 3(5)
C(7)	0.420 8(3)	0.595 9(2)	0.850 6(5)
C(8)	0.503 9(3)	0.523 7(2)	0.871 5(6)
C(9)	0.565 3(3)	0.505 7(2)	0.686 9(5)
C(10)	0.653 0(3)	0.565 8(2)	0.628 2(6)
C(11)	0.370 9(3)	0.619 9(2)	1.048 9(5)
C(12)	0.321 5(4)	0 546 7(3)	1.156 8(7)
C(13)	0.287 8(4)	0.689 2(2)	1.020 4(8)
C(14)	0.579 8(4)	0.419 1(2)	0.614 8(7)
C(15)	0.474 6(4)	0.817 8(2)	0.357 9(6)

Table 9. Bond lengths (Å) and angles (°) involving the non-hydrogen atoms for compound (4) (e.s.d.s in parentheses).

Bond length			
O(1)–C(10)	1.221(4)	C(5)–C(6)	1.322(5)
O(2)–C(14)	1.445(5)	C(6)–C(7)	1.505(4)
O(2)–C(9)	1.446(4)	C(7)–C(11)	1.537(5)
C(1)–C(2)	1.317(5)	C(7)–C(8)	1.551(5)
C(1)–C(10)	1.483(5)	C(8)–C(9)	1.498(5)
C(2)–C(3)	1.498(5)	C(9)–C(14)	1.485(5)
C(3)–C(4)	1.516(5)	C(9)–C(10)	1.503(5)
C(4)–C(15)	1.320(5)	C(11)–C(12)	1.513(5)
C(4)–C(5)	1.473(4)	C(11)–C(13)	1.524(6)
Bond angle			
C(14)–O(2)–C(9)	61.8(2)	C(11)–C(7)–C(8)	111.7(3)
O(2)–C(9)–C(14)	59.1(2)	C(7)–C(8)–C(9)	113.5(3)
O(2)–C(14)–C(9)	59.1(2)	O(2)–C(9)–C(8)	115.5(3)
C(2)–C(1)–C(10)	124.5(4)	O(2)–C(9)–C(10)	114.6(3)
C(1)–C(2)–C(3)	125.7(4)	C(14)–C(9)–C(8)	121.4(3)
C(2)–C(3)–C(4)	109.5(3)	C(14)–C(9)–C(10)	115.2(3)
C(15)–C(4)–C(5)	122.5(3)	C(8)–C(9)–C(10)	117.6(3)
C(15)–C(4)–C(3)	122.0(4)	O(1)–C(10)–C(1)	122.8(4)
C(3)–C(4)–C(5)	115.3(3)	O(1)–C(10)–C(9)	119.0(3)
C(4)–C(5)–C(6)	126.0(3)	C(1)–C(10)–C(9)	118.1(4)
C(5)–C(6)–C(7)	125.9(3)	C(12)–C(11)–C(13)	111.0(4)
C(6)–C(7)–C(11)	112.7(3)	C(12)–C(11)–C(7)	113.3(3)
C(6)–C(7)–C(8)	108.4(3)	C(13)–C(11)–C(7)	109.8(3)

($\rho = 0.03$) to downweight the intense reflections. Non-hydrogen atomic co-ordinates are listed in Table 5, and bond lengths and angles of the non-hydrogen atoms in Tables 6 and 7,* respectively.

X-Ray Molecular Structure Analysis of Compound (4).—A colourless parallelepiped crystal of compound (4) having dimensions of 0.15 × 0.20 × 0.40 mm was mounted on a glass fibre. The data were collected at 23 ± 1 °C on a Rigaku AFC5R diffractometer with graphite-monochromated Cu-K_α radiation and a 12 kW rotating anode generator. The crystallographic data for compound (4) are summarized in Table 4. The structure of compound (4) was solved by direct methods using DIRDIF and MITHRIL [22] programs. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined with assigned isotropic thermal parameters which were greater than the B_{equiv}-value of the atom to which they were bonded. The final cycle of full-matrix least-squares refinement converged with unweighted and weighted agreement factors of $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = \{[\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2]\}^{\frac{1}{2}}$. The standard deviation of unit weight was 1.18. The weighting scheme was based on counting statistics and included a factor ($\rho = 0.05$) to downweight the intense reflections. Non-hydrogen atomic co-ordinates were given in Table 8, and bond lengths and angles of the non-hydrogen atoms are in Table 9.†

Acknowledgements

We are grateful to Professor H. Hauptmann of the University of Regensburg for sending X-ray crystallographic data of (±)-periplanone-B (1). We thank Dr. Y. Takagi of T. Hasegawa Co. Ltd. for the gift of germacrene-D (7). We also thank Mr. T. Tobita of Tobacco Science Research Laboratory, Japan Tobacco Inc. (JT) for recording the NMR spectra. Special thanks are due to Dr. Y. Kitano of Toray Research Centre for the X-ray crystallography measurements.

References

1 C. J. Persoons, F. J. Ritter, and W. J. Lichtendonk, *Proc. Kon. Ned. Akad. Wetensch. Amsterdam*, 1974, **C77**, 201 (*Chem. Abstr.*, **81**, 88 209f).
2 T. Kitahara, M. Mori, and K. Mori, *Tetrahedron*, 1987, **43**, 2689, and references cited therein. For recent progress on the synthesis of compound (1), see: S. Kuwahara and K. Mori, *Heterocycles*, 1989, **28**, 167, and references cited therein.
3 C. J. Persoons, P. E. J. Verwiel, F. J. Ritter, and W. J. Nooyjen, *J. Chem. Ecol.*, 1982, **8**, 439, and references cited therein.
4 H. Hauptmann, G. Mühlbauer, and H. Sass, *Tetrahedron Lett.*, 1986, **27**, 6189.
5 T. L. Macdonald, C. M. Delahunty, and J. S. Sawyer, *Heterocycles*, 1987, **25**, 305.
6 Y. Shizuri, K. Matsunaga, and S. Yamaura, *Tetrahedron Lett.*, 1989, **30**, 3693.
7 K. Mori and Y. Igarashi, *Tetrahedron Lett.*, 1989, **30**, 5145.
8 S. Kuwahara and K. Mori, submitted for publication in *Tetrahedron Lett.* This work was presented in part at the annual meeting of the Agricultural Chemical Society of Japan, Niigata, April, 1989 (*Abstracts*, p. 525).
9 S. Kuwahara and K. Mori, *Tetrahedron Lett.*, 1989, **52**, 7447.
10 S. Tahara, M. Yoshida, J. Mizutani, C. Kitamura, and S. Takahashi, *Agric. Biol. Chem.*, 1975, **39**, 1517; S. Takahashi, C. Kitamura, and I. Horibe, *ibid.*, 1978, **42**, 79.
11 W. S. Bowers and W. G. Bodenstein, *Nature*, 1971, **232**, 259; C. Nishino, K. Kobayashi, S. Manabe, and A. Mori, *Comp. Biochem. Physiol.*, 1989, **92A**, 129, and references cited therein.
12 W. C. Still, L. J. MacPherson, T. Harada, and J. F. Callahan, *Tetrahedron*, 1984, **40**, 2275; W. C. Still, *J. Am. Chem. Soc.*, 1979, **101**, 2493.
13 K. Okada, M. Mori, K. Shimazaki, and T. Chuman, *J. Chem. Ecol.*, in the press.
14 K. Okada, M. Mori, S. Kuwahara, T. Kitahara, K. Mori, K. Shimazaki, and T. Chuman, *Agric. Biol. Chem.*, 1990, **54**, 575.
15 C. Nishino and K. Kuwabara, *Comp. Biochem. Physiol.*, 1983, **74A**, 909; S. Takahashi, H. Takegawa, T. Takahashi, and T. Doi, *J. Pesticide Sci.*, 1988, **13**, 501.
16 H. Hauptmann, G. Mühlbauer, and N. P. C. Walker, *Tetrahedron Lett.*, 1986, **27**, 1315.
17 S. L. Schreiber and C. Santini, *J. Am. Chem. Soc.*, 1984, **106**, 4038.

* Non-hydrogen atom thermal parameters and hydrogen atomic co-ordinates calculated assuming ideal geometries have been deposited at the Cambridge Crystallographic Data Centre. For details see section 5.6.3 of the Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, in the January issue.

† Non-hydrogen atom thermal parameters and hydrogen atomic co-ordinates have been deposited at the Cambridge Crystallographic Data Centre.

18 For a recent review, see; J. Boeckh and K.-D. Ernst, *J. Comp. Physiol.*, 1987, **A161**, 549.
19 D. Neuhaus, *J. Magn. Reson.*, 1983, **53**, 199; M. Kimms and J. K. M. Sanders, *ibid.*, 1984, **56**, 518.
20 G. M. Sheldrick, SHELX-86, A Program for the Solution of Crystal Structure from Diffraction Data, Institute für Anorganische Chemie der Universität, Tammannstrasse 4, Göttingen, Federal Republic of Germany.
21 P. T. Beursken, DIRDIF, Direct Methods for Difference Structures – an Automatic Procedure for Phase Extension and Refinement of Difference Structure Factors, Technical Report 1984/1, Crystallography Laboratory, Toernooiveld, 6525 Ed Nijmegen, Netherland.
22 C. J. Gilmore, MITHRIL, An Integrated Direct Methods Computer Program, University of Glasgow, Scotland; see also *J. Appl. Crystallogr.*, 1984, **17**, 42.

Paper 9/05008F
Received 23*rd November* 1989
Accepted 8*th February* 1990