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Structure and Receptor Participation of Periplanone A, the Sex Pheromone of the American Cockroach

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Periplanones A (24 μg) and B (15 μg), the sex pheromones of the American cockroach, were isolated from the materials derived from 11500 females. The structure of periplanone A was elucidated to be (1Z,5E,7S*, 10R*)-10(14)-epoxy-1,4(15),5-germacratrien-9-one. Either pheromone was revealed to participate with the corresponding sex pheromone receptor.

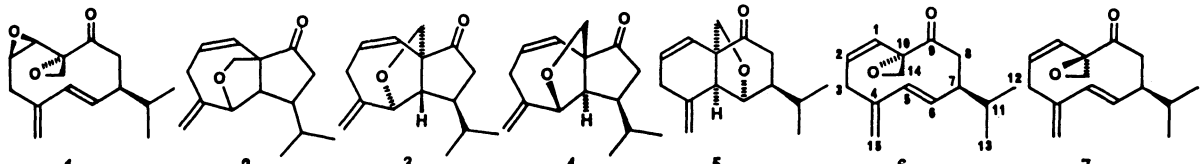
For the structures of periplanones A (PA) and B (PB), the sex pheromones of the American cockroach (Periplaneta americana), although the germacradienone dioxide structure (**1**) is established for PB,[1] the structure of PA is now confusable among a selinadienone (**5**) and germacratrienone oxides (**6** and **7**).

For the structure of PA, the first hydroazulenone structure (**2**)[2] was negated by the spectral data of synthetic isomers (**3** and **4**) of **2**,[3] and the structure **5**[4] was speculated from molecular mechanics calculation data and the ^{1}H NMR data reported for **2**.[2a] Recently, germacrene type structures, **6** and **7**, were proposed to natural[5] and synthetic[6] PA, respectively. Hence, in this study we aim to confirm the structure of natural PA.

Two sex pheromones, pheromones A (24 μg) and B (15 μg)(both active at 10^{-4} μg dose level), were isolated from the CH_2Cl_2 extract (110 g) of the filter papers dirted by 11500 females of the insect and their faeces collected for 5 years, by the previously reported procedure monitored by male electroantennogram (EAG) responses.[7]

The homogeneity of the pheromones was guaranteed by GC analysis[8] in which both pheromones partially changed to other compounds at more than 150 °C. The molecular formulae of pheromones A and B were assigned to $C_{15}H_{20}O_2$ [M^+, m/z 232; exact MS: m/z 232.1452 (Δmmu -1.1)] and $C_{15}H_{20}O_3$ (M^+, m/z 248) from their MS, respectively. Since the MS of pheromones A and B were respectively identical with those of PA and PB reported previously,[2b,7] pheromones A and B are expressed as PA and PB, respectively, hereafter.

UV (hexane) and IR (KBr) absorbances of PA [λ_{max}: 220 nm and ν_{max}: 1710, 1632, 1385, 1370 cm^{-1}] showed the presence of diene conjugated system, ketone and isopropyl groups.



1 **2** **3** **4** **5** **6** **7**

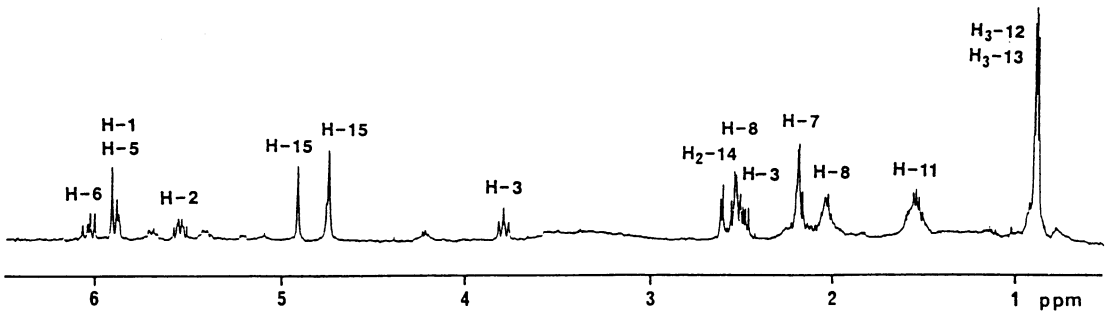


Fig. 1. ^{1}H NMR spectrum of PA in CS_2 (24 μg in 0.4 ml) at 400 MHz.

Since the ^{1}H NMR spectrum of Persoons' PA has been taken in CS_2,[2a] the spectrum of PA was measured in the same solvent (400 MHz, 27 °C)(Fig. 1), but it was different from that of Persoons' PA.[2a] Although the ^{1}H NMR was repeatedly measured every 1 h for 5 h, no signal change was observed during the period meaning stable property of PA.

In the ^{1}H NMR spectrum, chemical shifts, splitting patterns and coupling constants of the olefinic proton signals at δ5.54 (1H, ddd, J=11.2, 10.7, 7.3 Hz), 5.89 (1H, d, J=11.2 Hz), 5.88 (1H, d, J=16.1 Hz) and 6.03 (1H, dd, J=16.1, 10.7 Hz) showed the presence of the groups, $-CH_2-\overset{H}{C}=\overset{H}{C}-C-$ and $-C-C=\underset{H}{C}-CH$. The methylene protons of the former group resonated at δ2.49 (1H, dd, J=10.7, 7.3 Hz) and 3.78 (1H, t, J=10.7 Hz) allowed to expand further the group to $-C-CH_2-\overset{H}{C}=\overset{H}{C}-C-$.

Rather low field resonance and geminal coupling constant of one proton signal at δ2.53 (dd, J=11.2, 9.7 Hz) suggested that this proton was a component of a methylene group adjacent to the ketone group. Furthermore, double doublet splitting of this proton showed the presence of the group, $HC-CH_2-CO-$. Another proton of the methylene group may be a signal at δ2.04 (1H, m) or 2.19 (1H, m).[9]

Proton signals of an isopropyl group [$HC-CH(CH_3)_2$] appeared at δ0.88 (3H, d, J=6.8 Hz), 0.89 (3H, d, J=6.8 Hz), 1.55 (1H, m) and 2.04 (1H, m) or 2.19 (1H, m).[9] A 2,2-disubstituted oxiran ring is contained in PA because of signals at δ2.53 (1H, d, J=5.9 Hz) and 2.61 (1H, d, J=5.9 Hz). Protons of an exo methylene group resonated at δ4.72 (1H, s) and 4.89 (1H, s), and this group was expected to be a component of the diene conjugated system.

From the molecular formula and the above partial structures, a germacratrienone oxide structure such as **6** or **7** was assignable to PA.

In order to compare ^{1}H NMR data among PA, **6**[5] and **7**,[6] the spectrum of PA was taken in $CDCl_3$ (500 MHz, 27 °C) after recovery of PA from the CS_2 solution. The ^{1}H NMR data and proton assignments are listed in Table 1 together with those of **6** and **7**. As seen in Table 1, the ^{1}H NMR data were rather different between **6** and **7**, and the data of PA were almost identical with those of **6** whose stereochemistry has been established by synthetic work of Hauptmann et al.[5] Thus, the structure of PA was confirmed to be **6**.

The receptor participation of PA and PB has been elucidated by the differential saturation EAG (DS-EAG) technique,[10] before the structure of PA was confirmed. However, for verifying the genuineness of PA or an artifact compound, for example, from PB, the receptor participation of PA and PB should be reinvestigated. The DS-EAG technique is applied here for the purpose. In the DS-EAG, a successfully saturated an antennal sex pheromone receptor of the male by a high concentration of a pheromone does not respond to

Table 1. ^{1}H NMR data and proton assignments of PA, **6** and **7**

Proton	PA[a]	6[b]	7[c]
H-1	5.95(d,11.0)[d]	5.98(d,10.0)	6.05(d,11.2)
H-2	5.64(ddd,11.0,10.6,7.4)	5.66(ddd,11.3,10.0,7.3)	5.95(ddd,11.2,8.5,3.5)
H_2-3	2.57(dd,11.9,7.4)	2.6[f]	2.80(dd,12.4,3.5)
	3.74(dd,11.9,10.6)	3.7[f]	3.14(dd,12.4,8.5)
H-5	5.96(d,16.1)	5.85(d,16.0)	5.87(d,15.0)
H-6	6.07(dd,16.1,10.6)	6.10(dd,16.0,10.0)	5.68(dd,15.0,6.5)
H-7	2.11(m)	2.1[f](ddd,10.0,9.6,5.5)	2.35(m)
H_2-8	2.08(m)	2.1[f]	2.53(m)
	2.66(t,10.2)	2.7[f]	
H-11	--[e]	1.6[f]	1.70(m)
H_3-12	0.88(d,6.7)	0.9[f]	0.87(d,6.7)
H_3-13	0.90(d,6.7)		0.93(d,6.7)
H_2-14	2.83(d,5.4)	2.87(d,5.4)	2.90(d,6.0)
	2.87(d,5.4)	2.87(d,5.4)	3.08(d,6.0)
H_2-15	4.76(s)	4.78(m)	4.82(s)
	4.95(s)	4.95(m)	4.95(s)

a) Measured in $CDCl_3$ at 500 MHz (24 μg in 0.4 ml). b) The data are from Ref. 5 ($CDCl_3$, 360 MHz). c) The data are from Ref. 6 ($CDCl_3$, 360 MHz). d) All data are represented as a set of chemical shift (δ), coupling pattern and coupling constant (J in Hz). e) Unreadable signal. f) Read by us from the ^{1}H NMR chart in Ref. 5.

further (secondary) stimulation with the same pheromone, but other sex pheromone receptor(s) still respond to other sex pheromone(s). Therefore, mutual saturation and secondary stimulation with the pheromones can elucidate receptor participation of them.

DS-EAG responses were recorded using 1 x 10^{-2} μg and 3 x 10^{-3} μg of the isolated pheromones for the saturation and secondary stimulation, respectively, and are shown in Fig. 2. In the antennae saturated by PA (Fig. 2A), PB evoked significant secondary response (b/A in Fig. 2A), but PA negligible response [a/A, compare with the control response by air (c/A)]. This means that PB interacts with the PB-receptor. In the case of saturation by PB (Fig. 2B), PA gave significant secondary response (a/B), while PB actually no response [b/B, (c/B, control)], meaning the interaction of PA with the PA-receptor. These results were reproducible in 3 repetitions.

The fact that PA and PB participate with the corresponding sex pheromone receptors indicates the genuineness of both pheromones and precise recognition mechanism of the receptors between similar structures, **6** and **1**.

As reported previously,[4] the ^{1}H NMR data of Persoons' PA[2a] are explainable the structure **5** which is assumed to be unstable.[4] In contrast to labile property of Persoons' PA[2a] (even at -20 °C), our PA is stable at 27 °C. However, it becomes unstable at more than 150 °C (GC). Persoons' PA (**5**) is, hence, suggested to be derived from **6** artificially, especially in the preparative GC in Persoons' isolation

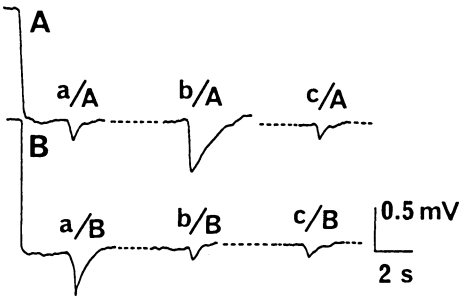


Fig. 2. DS-EAG responses from male antennae of the American cockroach.
A and B represent responses saturated by 1 x 10^{-2} μg of PA and PB, respectively. Secondary responses are elicited by 3 x 10^{-3} μg of PA (a/A and a/B) and PB (b/A and b/B). c/A and c/B are control responses by air only.

procedure.[11] Moreover, Persoons' PA could not be found in the female midgut[1a] (probably the organ for sex pheromone production), which also suggests that his PA is artifact.

PB (**1**) may be biogenetically derived from **6** which may convert to **5** artificially. The conversion of **6** to **5** involves the following simultaneous S_N2 type shifts: the 5,6-double bond to C-10 from β-side and the C-10-O bond to C-6 from α-side. For these rearrangements, the configuration of the epoxide ring of **6** is more reasonable than that of **7**.

In the synthesis of **7**, a possible contamination of **6** has been suggested.[6] The contaminant might cause the pheromonal activity of **7**.

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8) 4% OV-1, 3 mm (i.d.) x 2 m, 145 °C for PA (t_R: 8.15 min) and 150 °C for PB (t_R: 10.72 min), N_2 50 ml/min.
9) From the proton assignments in Table 1, the signal at δ2.04 was later assigned to H-8, and the signal at δ2.19 to H-7.
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