SYNTHETIC STUDIES OF PERIPLANONE A, A SEX PHEROMONE OF PERIPLANETA AMERICANA[Φ]

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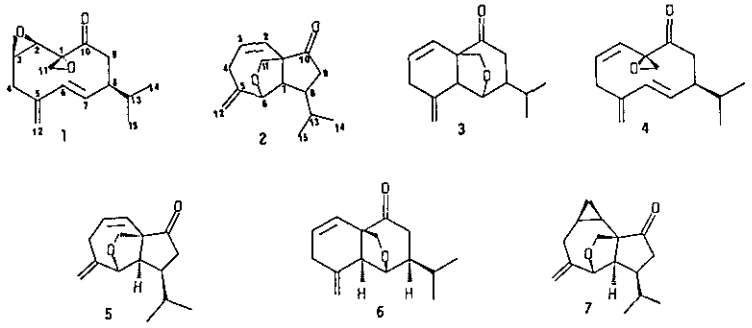
Abstract —— Germacratrienone oxide 4 is proposed to be periplanone A, the minor component of the sex pheromone of Periplaneta americana, and to undergo transannular cyclization to oxamethylene-bridged hydroazulenone 5, which had previously been postulated without stereochemical definition to be the natural pheromone.

The American cockroach, Periplaneta americana, has played an important role in the study of intra-species chemical communication. As early as 1952, an attractant from female cockroaches was shown to elicit intense excitement in males of the same Periplaneta species (1). In 1974, Persoons et al. isolated two highly active components of the natural pheromone, labeled periplanone A (≈20 μg, MW = 232) and periplanone B (≈200 μg, MW = 248), from an isolation program of more than 75,000 virgin females (2). Detailed spectroscopic analysis of these compounds led to the non-stereochemically defined structural proposals for periplanone B as germacrone diepoxide 1 (3,4) and periplanone A as the tricyclic sesquiterpene 2 (5,6). Confirmation of the structure for periplanone B and establishment of its absolute stereochemistry as indicated in 1 came in 1979 from synthetic studies of Still (7) and structural investigations of Nakanishi, Still et al. (8), culminating more than 25 years of work on the problem (9).

Much less is known about periplanone A, the minor component of the natural pheromone. Extensive ^{1}H NMR analysis indicated that the compound isolated by Persoons et al. was unstable with regard to rearrangement to a biologically inactive system proposed to be tetrahydrofuran-bridged octalin 3 (6). Recent electrophysiological studies have demonstrated that specialized receptors in the antennae and specific neurons in the brain of Periplaneta males exist which enable them to differentiate between periplanone A and B (10-14). Thus, the natural pheromone appears to be composed of both compounds, although detailed understanding of the behavioral and physiological roles that each component plays has not emerged (15,16).

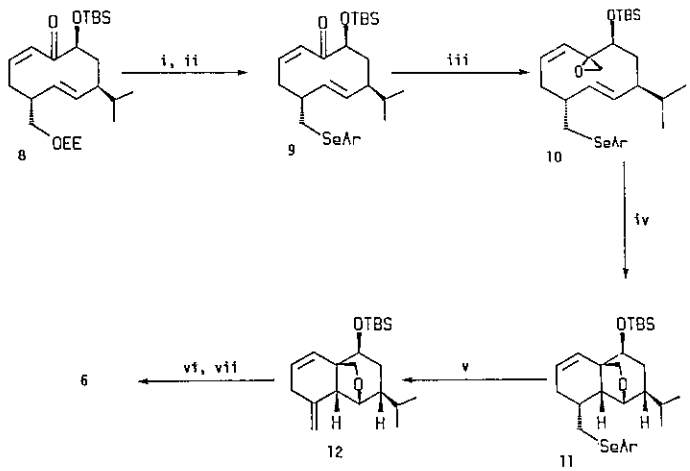
[Φ]Dedicated to Professor Gilbert Stork on the occasion of his sixty-fifth birthday.

Our goals have been to determine the structure of periplanone A through synthesis and to use this structural information to define the biosynthetic relationships between the periplanones A and B and elucidate the essential pharmacophores of the individual pheromones for interaction with their highly specific receptors. Our synthetic studies lead us to propose germacrone oxide 4 as the structure for periplanone A and to assign the tricyclic structure 5 to the material isolated by Persoons et al., which we postulate to be a physiologically active artifact of their isolation procedure.

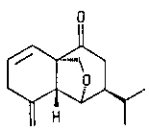


Our structural proposal for periplanone A evolved from our syntheses of octalin 6 and cyclopropyl hydroazulenone 7. Octalin 6 was available in 7 steps (18% yield) from germacrone 8, an intermediate in Still's synthesis of periplanone B (7) (Figure 1). Thus, hydrolysis of the ethoxyethyl protective group of 8 and conversion of the alcohol to the selenide gave 9, which was epoxidized to afford germacradiene 10. We have been unable to unambiguously confirm the stereochemical orientation of the epoxide in germacradiene 10 on the basis of spectral data alone, although we believe that the relative stereochemistry of the epoxide center results from exo-face approach to the germacranone system affording the epi-periplanone B stereochemistry on the basis of molecular modelling and by analogy with additions to structurally analogous epoxy germacrone systems [see Still (7)]. Under mild acid conditions, germacradiene 10 underwent smooth transannular cyclization to provide a tricyclic structure consistent with either the octalinyl or hydroazulenyl carbocyclic framework. Establishment of the octalinyl carbocyclic system 11 was obtained by conversion of 11 into octalone 6 via the intermediacy of octalin 12. Complete stereochemical definition of octalone 6 was obtained by first order ^{1}H NMR analysis at 360 MHz (Figure 2). Using octalin 6 as a spectral template, confirmation was achieved of the trans-octalin systems of intermediates 11 and 12 and thereby of the mechanism of transannular epoxy-olefin cyclization as proceeding with inversion of stereochemistry at the epoxide center.

Figure 1. Synthesis of Tetrahydrofuran-Bridged Octalone 6



REACTION CONDITIONS: (i) AcOH, MeOH (86%); (ii) o-NO_2-PhSeCN, Bu_3P, THF (85%); (iii) $Me_3S^+I^-$, KH, DMSO/THF (62%); (iv) TFA, CH_2Cl_2, 0°C (66%); (v) H_2O_2, THF (95%); (vi) TBAF, THF, 0°C (90%); (vii) CrO_3, pyr (75%).



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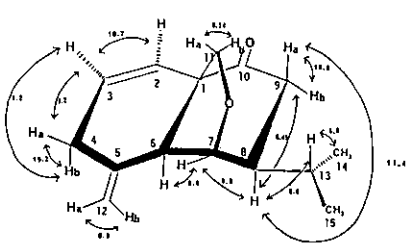
It was apparent that the periplanone A rearrangement product (3) was the isopropyl epimer of our synthetic material and possessed structure 13 by comparison of the spectrum of octalone 6 with the published spectrum of the naturally derived octalone 3. Since stereochemical inversion of either the isopropyl group or the oxamethylene moiety of the tetrahydrofuran ring would not be expected to occur upon Wagner-Meerwein rearrangement of the carbocyclic skeleton of 2 to 3 based on mechanistic considerations, these studies served to establish a trans relative stereochemistry of these groups in the proposed structure for periplanone A.

Figure 2. Proton NMR Spectral Features of Octalone 6

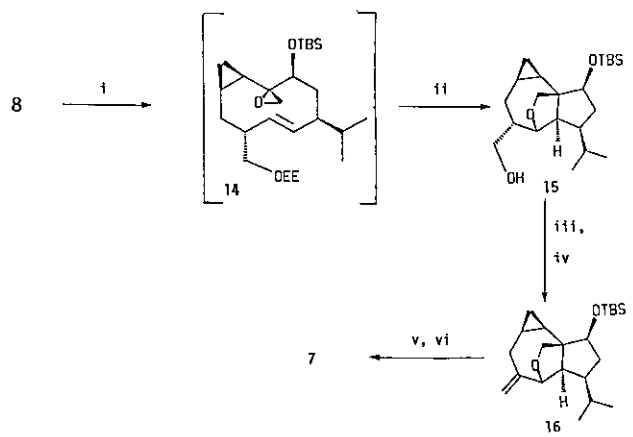
^{1}H NMR CHEMICAL SHIFT ASSIGNMENTS FOR OCTALONE 6

Proton	Chemical Shift (δ)
H-2	5.90
H-3	5.83
H-4a	2.90
H-4b	2.78
H-6	2.58
H-7	4.86
H-8	1.45
H-9a	2.33
H-9b	2.52
H-11a	3.95
H-11b	3.65
H-12a	5.12
H-12b	
H-13	1.78
H-14	0.92, 1.05
H-15	



We next set out to determine the ring junction stereochemistry of 2 relative to the oxamethylene bridge. Accomplishment of this goal became possible as a consequence of a crucial observation concerning the epoxidation of germacradienone 8 with dimethylsulfonium methylide (Figure 3). During epoxidation, a minor species in addition to the desired epoxide was obtained (≈1:2), which underwent facile transannular cyclization upon attempted hydrolysis of the ethoxyethyl protective group. Extensive spectroscopic analysis revealed the (most consistent) structure to be tetracycle 15 of undetermined relative cyclopropane stereochemistry. The chemical lability of the cyclopropyl germacrene oxide precursor 14 precluded its isolation in pure state and physical characterization, although we believe the relative epoxide stereochemistry to be epi (as depicted in 14) by the rationale argued for germacradiene oxide 10 above. If the stereochemical assignment of germacrone 14 is correct, transannular cyclization to 15 must proceed with inversion of stereochemistry. Determination of the cyclopropane stereochemistry and establishment of the hydroazulene structure of tetracyclic alcohol 15 was confirmed by conversion to the cyclopropane-containing analog of the proposed periplanone A system 7. This transformation was accomplished by conversion of the alcohol moiety into the corresponding selenide and oxidative elimination to produce exocyclic olefin 16, followed by silyl ether deprotection and oxidation to generate 7. The difference in the modes of cyclization of epoxy germacradienes 10 and 14 demonstrates clearly that the factors are delicately balanced which control the cyclization of these epoxy germacrane systems into decalinyl and hydroazulenyl ring systems.

Figure 3. Synthesis of Cyclopropyl Tetrahydrofuran-Bridged Hydroazulenone 7



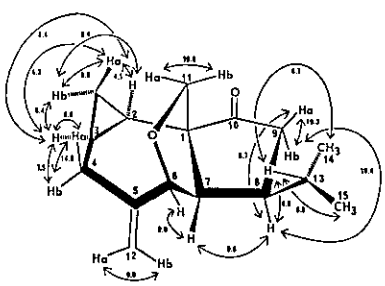
REACTION CONDITIONS: (i) $Me_3S^+I^-$, KH, DMSO/THF; (ii) AcOH, MeOH (24% from 9); (iii) o-NO_2-PhSeCN, Bu_3P, pyr (81%); (iv) H_2O_2, THF (83%); (v) TBAF, THF, 0°C (94%); (vi) CrO_3, pyr (79%).

The ^{1}H NMR spectrum of cyclopropyl hydroazulenone 7 could be completely assigned (Figure 4). The β-orientation of the cyclopropane group relative to the oxamethylene bridge was assigned on the basis of a nuclear Overhauser effect between the endo-C_{2a} proton and one of the ether bridge (C_{11}) protons. However, a critical difference between 7 and the published spectrum of periplanone A 2 (6) was observed; in the spectrum of cyclopropyl hydroazulenone 7, the C_6 proton is a singlet (4.73 δ) at 360 MHz, whereas the C_6 proton is a doublet (J=-4.5 Hz; 4.54 δ) at 300 MHz in the natural product. Although this spectral difference could be rationalized by several alternatives, including incorrect assignment of structure to the natural product or perturbation of the spectrum of 7 relative to periplanone A by the cyclopropane group, the most consistent possibility was that the proposed structure for periplanone A possessed a cis-hydroazulene ring fusion, which therefore requires the incorporation of a trans oxabicyclo[3.3.0]octane unit into the naturally-derived material. The difference in the ^{1}H NMR coupling pattern for the C_6 proton would thus be resolved since the trans-fused oxabicyclo[3.3.0]octane system would exhibit the required C-6/C-7 coupling constant, as determined by molecular mechanics calculations (17). In addition, a fundamental structural difference between the natural system and 7 was implicated by the marked stability of 7 to hydroazulenone--octalone rearrangement, relative to the noted sensitivity of the compound isolated by Persoons et al., although other differences in the two structures could underlie this chemical distinction [such as the presence of the cyclopropane ring in 7 or the steric compression postulated to be a consequence of the isopropyl stereochemistry of the natural material (see below)].

Figure 4. Proton NMR Spectral Features of Hydroazulenone 7

^{1}H NMR CHEMICAL SHIFT ASSIGNMENTS FOR CYCLOPROPYL HYDROAZULENONE 7

Proton	Chemical Shift (δ)
H-2	1.00
H-2aa	0.59
H-2ab	0.89
H-3	0.97
H-4a	1.86
H-4b	2.66
H-6	4.73
H-7	2.45
H-8	1.92
H-9a	2.81
H-9b	2.16
H-11a	3.74
H-11b	3.79
H-12a, H-12b	4.78, 4.82
H-13	1.69
H-14, H-15	0.96, 1.07



Therefore, by integration of the conclusions of our structural studies on octalone 6 and hydroazulenone 7 and by analogy with the absolute stereochemistry of periplanone B, we propose that the material assigned to be periplanone A by Persoons et al. has structure 5. This structure is exceptional in that it represents an example of a rare trans-fused oxabicyclo-

[3.3.0]octane system (18), which exhibits significant additional steric strain due to the gauche-gauche pentane disposed isopropyl and exo-methylene units of the carbocyclic framework; the total strain energy amounts to approximately 19 kcal/mol relative to the cis-fused ring system (17). The hydroazulenone--octalone rearrangement observed in the natural series, which we propose to be of structure 5 to 13, would thus proceed through a mechanism involving retention of stereochemistry at the C_6 and C_7 rearrangement termini, as well as at the ring-junction migratory carbon (C_1).

The formation of such highly strained ring systems has ample precedent in Nature, although the relief of the strain energy of 5 via the agency of an allylic carbocation would rationalize the facile isomerization of the hydroazulenyl system into the octalinyl system noted by Persoons et al. However, literature debate concerning the stability of periplanone A (cf. 10,11), which appeared a priori to exhibit a dependency on the isolation technique, led us to consider the possibility that a precursor to hydroazulenone 5 was the actual pheromone and that hydroazulenone 5 was derived from either thermally-induced or acid-catalysed transannular cyclization during the course of (gas chromatographic) isolation by Persoons et al. (2-6). We therefore turned our attention to synthesis of the possible cyclodecatrienone oxide precursors of hydroazulene 5--isomeric germacratrienone oxides 4 and 18.

The mode of cyclization and the possible biosynthetic relationships for conversion of cyclodecatrienone oxides 4 and 18 into periplanone B 1 and the proposed tricyclic structure 5, which either is a rearrangement product of the natural pheromone or is periplanone A, are depicted in Figure 5. The germacratrienone oxide precursor with the epi-C_1 epoxide stereochemistry relative to periplanone B could produce 5 via transannular cyclization with retention of the epoxide stereochemistry, whereas 2,3-des-epoxy periplanone B 18 could undergo cyclization to 5 via a process involving inversion of the epoxide stereochemistry. Our studies of the cyclization of epi-C_1-periplanone B epoxides have indicated that inversion of epoxide stereochemistry is the overwhelming mode of reaction under acid-catalysed conditions. However, related studies of the cyclization of substrates with the periplanone B epoxide stereochemistry have indicated that such compounds were remarkably stable to acid-promoted transannular cyclization relative to the epi-epoxide configuration. Moreover, the process of cyclization--thermal, acid-catalysed, or potentially enzymatic--could have a significant impact on the mechanism of cyclization and thus its stereochemical outcome and is unknown. Therefore, our efforts focussed on the synthesis of germacratrienone oxide 4, in order to assess its potential to be the putative precursor to 5 or to be the natural pheromone. Our approach proceeded by oxidative elimination of the selenide in 10 to produce germacratriene oxide 19, which was desilylated and oxidized to germacrone 4 (Figure 6).

Figure 5. Proposed Transannular Cyclization of Cyclodecadienone Oxides 4 and 18 to Hydroazulenone 7

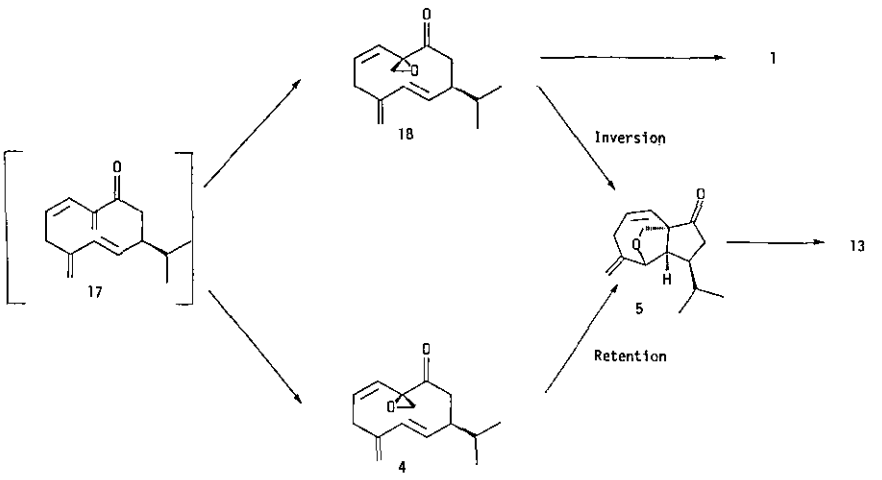
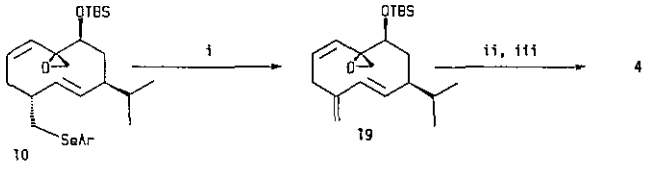


Figure 6. Synthesis of Germacratrienone Oxide 4, Periplanone A



REACTION CONDITIONS: (i) H_2O_2, THF (74%); (ii) TBAF, THF, 0°C (86%); (iii) CrO_3, pyr (62%).

Germacratrienone oxide 4 was fully consistent with its designated structure by spectroscopic analysis (19), although the epoxide stereochemistry has not been rigorously established (see above) and the synthetic material could contain traces (<-3%) of its epoxide stereoisomer (18) or the proposed cyclization product (5), which could confound bioassay. Despite these potential drawbacks, synthetic germacratrienone oxide 4 exhibited a lower threshold of activity at 10^{-7}-10^{-8} µg/ml by bioassay (15,16), which is essentially identical to periplanone B. Confirmation of germacrone oxide 4 as periplanone A must await electrophysiological studies of the interaction of 4 with the specific periplanone A receptor, since the possibility remains that germacrone 4 could act as a periplanone B mimic. Clearly, synthesis and biological evaluation of the structure proposed for periplanone A by Persoons et al., which we postulate to

be 5, and of germacratrienone oxide 18 must be undertaken in order to completely elucidate the intricate relationships between the chemistry, biosynthesis and physiological activities of periplanone B and these germacrone oxide and tricyclic sesquiterpene candidates for periplanone A. Nonetheless, the potent activity of germacrone oxide 4 is notable, given the extraordinary ligand selectivity that these receptors exhibit (cf. 7), and persuasively supports its presentation as periplanone A.

The relationships between the biosynthesis and the receptor specificity for the periplanones A and B are particularly intriguing, if germacrone oxide 4 is the natural pheromone. Our speculation concerning the biosynthesis of the pheromones (Figure 5) suggest that a tetraunsaturated germacrone precursor (17) would undergo epoxidation at the C_1-C_{11} olefin from either face, producing the epoxy germacrone oxides 4 and 18--one of which is periplanone A and the other of which is subsequently oxidized to become periplanone B. Differentiation of the two pheromones 1 and 4 at the level of the receptor--specific receptor recognition--must be a function of subtle differences in the conformational behaviors of these two closely related germacrone oxide species. Understanding the nature of the substrate specificities of these highly sensitive receptors will contribute in a fundamental way to our knowledge of ligand-receptor theory.

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19. Our tentative assignments for the protons in germacratrienone 4 are as follows (δ, $CDCl_3$, 360 MHz): C_2 (6.05 δ; d, J= 11.2); C_3 (5.59 δ; d,d,d, J= 11.2, 8.5, 3.5); $C_{4a,b}$ (3.14 δ; d,d, J= 12.4, 8.5) and (2.80 δ; d,d,, J= 12.4, 3.5); C_6 (5.87 δ; d, J= 15.0); C_7 (5.68 δ; d,d, J=15.0, 6.5); C_8 (2.35 δ, m); $C_{9a,b}$ (for both protons) (2.53 δ; m)); $C_{11a,b}$ (3.08 δ; d, J= 6.0) and (2.90 δ; d, J = 6.0); $C_{12a,b}$ (4.95 δ; s) and (4.82 δ; s); C_{13} (1.70 δ; m); and $C_{14,15}$ (0.93 δ; d, J= 6.7) and (0.87 δ; d, J= 6.7).

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