Volatile Compounds from Androconial Organs of Danaine and Ithomiine Butterflies

S. Schulz, W. Francke

Universität Hamburg, Institut für Organische Chemie, Martin-Luther-King-Platz 6,
D-2000 Hamburg 13, Bundesrepublik Deutschland

J. Edgar

CSIRO, Division of Animal Health, P.O. Parkville, Vic. 3052, Australia

D. Schneider

Max-Planck-Institut für Verhaltensphysiologie, D-8131 Seewiesen, Bundesrepublik Deutschland

Z. Naturforsch. **43c,** 99–104 (1988); received July 29, 1987

Pyrrolizidine Alkaloids, Oxoisophorone, Bishomoterpenes, Butterfly-Pheromones, Danaines and Ithomiines, Buddleja Flowers

Pyrrolizidine alkaloid derivatives are present in the androconial (male scent) organs of *Prittwitzia hymenaea, Mechanitis isthmia veritabilis, Tithorea harmonia furia* (Lep., Ithomiinae), *Amauris echeria* and *Euploea sylvester* (Lep., Danainae). While the ithomiines contain the new pyrrolizidine alkaloid derivative methyl hydroxydanaidoate, the danaines contain the known derivatives danaidone and hydroxydanaidal. In addition, 2,2,6-trimethyl-2-cyclohexen-1,4-dione (oxoisophorone) and related terpenoids have been identified from *Amauris, Euploea* and *Prittwitzia* as well as from the flowers of *Buddleja davidii* (Loganiaceae). Three new naturally occurring bishomoterpenes as well as mono- and sesquiterpenes and aromatic compounds add to the formation of species specific blends in the scent organs of the butterflies.

Introduction

The closely related Danainae and Ithomiinae (Nymphalidae) are tropical butterflies known for their complex pheromone biology [1] and their mimicry. The male scent-producing organs (androconia) of Danainae consist of abdominal hairbrushes ("hairpencils") and alar pocket- or patch-like glands, while male Ithomiinae possess fringes of long hairs at the costal edge of the hindwings. Male and female danaine and ithomiine butterflies are known to accumulate pyrrolizidine alkaloids (PAs) from plants [1]. The males metabolize these to nitrogen containing volatiles (dihydropyrrolizines) and to branched aliphatic acid derivatives which are secreted by the androconial organs [1]. It appears that these compounds are important cues for sexual and/or social communication. The androconial organs are used differently in the Danainae and Ithomiinae. In most danaine genera (*Amauris, Danaus, Ideopsis* and *Tirumala*) male hairpencils are only expanded immediately prior to mating [1], however, in *Euploea* they are also displayed for longer periods during patrolling flights. In the case of the Ithomiinae, bioassays by Haber [2] have provided evidence that the secretion of the alar fringes cause cross-attraction of ithomiines of both sexes and of different species and are thus responsible for the formation of multispecies assemblies (leks? [1]). Pliske, however, has obtained contrasting results which indicate inter- and intraspecies male repellency associated with the fringes of a number of ithomiine species and he has proposed that, in some species, components in the androconial secretions serve as male territorial-recognition pheromones and allomones [3].

Materials and Methods

The androconial organs were obtained from butterflies collected in the field: 200 *Amauris echeria* (Stoll) (Danainae) from Kenya, East Africa, 15 *Euploea sylvester* (F.) (Danainae) from Sri Lanka and 50 *Prittwitzia hymenaea* (Prittwitz) (Ithomiinae) from North-Argentine. Danaine hairpencils were artificially protruded and excised, ithomiine fringes were cut-off with scissors. All organs were stored in pentane (Merck, Uvasol). These extracts were concentrated to ca. 30 μl and analyzed by GC and GC/MS without preseparation of high boiling lipids. In addition, methylene chloride extracts of the costal fringes

---

Reprint requests to Prof. Dr. W. Francke.

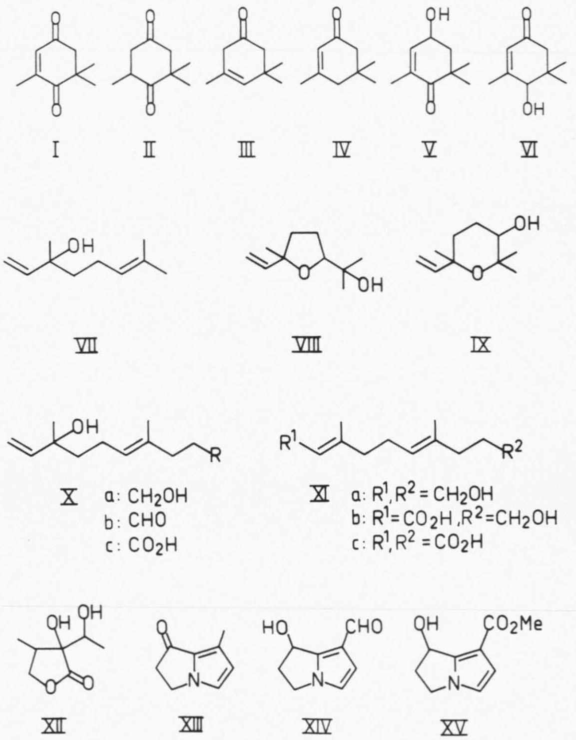
Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/88/0100–0099 $ 01.30/0

of 150 male *Mechanitis isthmia veritabilis* (Butler) (Ithomiinae) and 15 *Tithorea harmonia* (Cramer) (Ithomiinae) from Venezuela were analyzed. Flowers of *Buddleja davidii* Franch. (Loganiaceae) were collected at different locations near Hamburg (FRG) and aerated in a modified closed loop stripping system apparatus [4] equipped with a 1.5 mg charcoal filter as a trap. After 5 h the filter was extracted 3 times with 10 μl of carbondisulfide. The crude extract was submitted to GC and GC/MS. Gas chromatographic separations were carried out on a 60 m DB-5-CB capillary column programmed from 60−280 °C at a rate of 3 °C/min, a 50 m WG-11 fused-silica column programmed from 60−220 °C at a rate of 3 °C/min and on a 1 m × 2 mm, glass-lined stainless steel, 1% SE-30 packed column programmed from 70−220 °C at a rate of 6 °C/min. Mass spectra (70 eV) were obtained with a Varian MAT 311 A instrument connected to a Carlo-Erba 2150 gas chromatograph and on a Varian MAT 111 A mass spectrometer linked to a Varian 1400 gas chromatograph. Identification of natural compounds base on comparison of mass spectra and retention times with those of synthetic samples.

2,6,6-Trimethyl-2-cyclohexen-1,4-dione (oxoisophorone) was prepared from commercially available isophorone [5]. Reduction of oxoisophorone with lithiumaluminiumhydride gave 4-hydroxy-2,6,6-trimethyl-2-cyclohexenone and 4-hydroxy-3,5,5-trimethyl-2-cyclohexenone which could be separated by preparative GC on SE-30. 2,2,6-Trimethylcyclohexan-1,4-dione was formed by base catalyzed rearrangement of 3,4-epoxy-3,5,5-trimethyl-2-cyclohexanone (β-phoronoxide) [6]. (*E*)- and (*Z*)-8-Hydroxy-4,8-dimethyl-4,9-decadienal were synthesized from nerolidol according to known methods [7]; the corresponding dihydroxy compound and the respective hydroxy acid were obtained by reduction or oxidation respectively. Oxidation of the allylic alcohol, heliotridine, to the corresponding carboxylic acid methyl ester was achieved by Corey's method [8]. Further oxidation by manganese dioxide yielded 7-carbomethoxy-1,2-dihydro-(3H)-pyrrolizin-1-ol (methyl hydroxydanaidoate).

Results and Discussion

The androconial organs of the butterfly species investigated here emit bouquets of volatiles composed of acetogenins, aromatic compounds, a variety of terpenes, norterpenoids, bishomoterpenes and alkaloids. The compounds identified are compiled in Table I, chemical structures are shown in the formula scheme. Although the species investigated live on different continents their secretions were found to share closely related compounds obviously forming species specific compositions. Methyl hydroxydanaidoate (**XV**), (*E*)-4,8-dimethyl-4,9-decadien-1,8-diol (**Xa**), (*E*)-8-hydroxy-4,8-dimethyl-4,9-decadienal (**Xb**), and (*E*)-8-hydroxy-4,8-dimethyl-4,9-decadienoic acid (**Xc**) represent new natural products, while the following compounds are new insect volatiles: 2,2,6-trimethylcyclohexan-1,4-dione (**II**), 4-hydroxy-2,6,6-trimethyl-2-cyclohexenone (**V**), 4-hydroxy-3,5,5-trimethyl-2-cyclohexenone (**VI**), 2-ethenyl-5-(1-hydroxy-1-methylethyl)-2-methyltetrahydrofurane (**VIII**) and 2-ethenyl-5-hydroxy-2,6,6-trimethyltetrahydropyrane (**IX**). Several derivatives of PAs were found in the androconial organs of the investigated species. Such compounds are typical for Danainae and Ithomiinae, the males of which gather PAs as adults and use them for defence and as pre-



Formula scheme: Structures of identified compounds, see also text in Table I.

Table I. Volatile compounds identified from *Amauris echeria* (*A. ech.*, 10 replications), *Euploea sylvester* (*E. syl.*, 5 replications) and *Prittwitzia hymenaea* (*P. hym.*, 3 replications). x < 10 ng, xx = 10–200 ng, xxx > 200 ng per individual.

Compound	*A. ech.*	*E. syl.*	*P. hym.*
2-Hexenal		xx	
11-Dodecanolide		xx	
Benzaldehyde	x		
Phenylacetaldehyde	x		
Vanilline		x	
Eugenol	x	x	x
Methylsalicylate	x	xxx	
Anisol		x	
Oxoisophorone (**I**)	xxx	x	xx
2,2,6-Trimethylcyclohexan-1,4-dione (**II**)	xx	x	x
β-Phorone (**III**)	x		
Isophorone (**IV**)	x		
4-Hydroxy-2,6,6-trimethyl-2-cyclohexenone (**V**)		xxx	x
4-Hydroxy-3,5,5-trimethyl-2-cyclohexenone (**VI**)			xx
Linalool (**VII**)		xxx	
2-Ethenyl-5-(1-hydroxy-1-methylethyl)-2-methyltetrahydrofurane (**VIII**)	x		
2-Ethenyl-5-hydroxy-2,6,6-trimethyltetrahydropyrane (**IX**)	x		
(*E,E*)-α-Farnesene	x		
Nerolidol		x	
(*E*)-4,8-Dimethyl-4,9-decadien-1,8-diol (**Xa**)		xxx	
(*E*)-8-Hydroxy-4,8-dimethyl-4,9-decadienal (**Xb**)		xxx	
(*E*)-8-Hydroxy-4,8-dimethyl-4,9-decadienoic acid (**Xc**)		xxx	
2-Hydroxy-2-(1-hydroxyethyl)-3-methyl-γ-butyrolactone (**XII**)			xx
Danaidone (**XIII**)	xxx	xxx	
Hydroxydanaidal (**XIV**)	x	xxx	
Methylhydroxydanaidoate (**XV**)			xx

cursors for the biosynthesis of pheromone components [1, 9, 10]. A possible degradation product of pyrrolizidine alkaloids, 2-hydroxy-2-(2-hydroxyethyl)-3-methyl-γ-butyrolactone (**XII**), which was previously found in seven species of Ithomiinae from four different genera [11] proved to be present in rather large amounts also in *P. hymenaea,* along with traces of a diastereomer. In *A. echeria* danaidone (**XIII**) was previously identified [12]; we confirmed its presence and additionally found the related hydroxydanaidal (**XIV**). Both danaidone (**XIII**) and hydroxydanaidal (**XIV**) were also identified in hairpencils of *E. sylvester* [13]. In the ithomiines, *P. hymenaea, M. isthmia veritabilis* and *T. harmonia furia,* we found "methyl hydroxydanaidoate" (**XV**) in relatively large amounts. The mass spectrum of (**XV**) is shown in Fig. 1. The observed diagnostic ions derive from loss of water (*m/z* 163), loss of a methoxy group (*m/z* 150), loss of carbomethoxy (*m/z* 122) and subsequent loss of water (*m/z* 104). Several cyclohexanone derivatives were identified in *A. echeria, E. sylvester* and *P. hymenaea:* while oxoisophorone (**I**; 2,6,6-trimethyl-2-cyclohexene-1,4-dione) is a prominent compound in *A. echeria* and *P. hymenaea,* one of its reduction products (**VI**) was found as a major component in *P. hymenaea* exclusively. In contrast, another reduction product of oxoisophorone, (**V**), represents a major component in *E. sylvester*. In *P. hymenaea* we identified considerable quantities of the ketones (**I**) and (**VI**) as well as traces of eugenol and the ketones (**II**) and (**V**). Oxoisophorone (**I**) and isophorone (**IV**) have been previously identified as trace constituents of the defensive secretion of a grasshopper species (*Romalea* [14]), while isophorone and β-phorone (**III**) have been described as volatile constituents of females of the bark beetle, *Ips typographus* [15].

In the course of our studies on volatile constituents of flowers we analyzed the blossoms of *Buddleja davidii,* an ornamental garden bush, introduced to Europe from China. Its aromatic flowers are outstandingly attractive to butterflies and many other

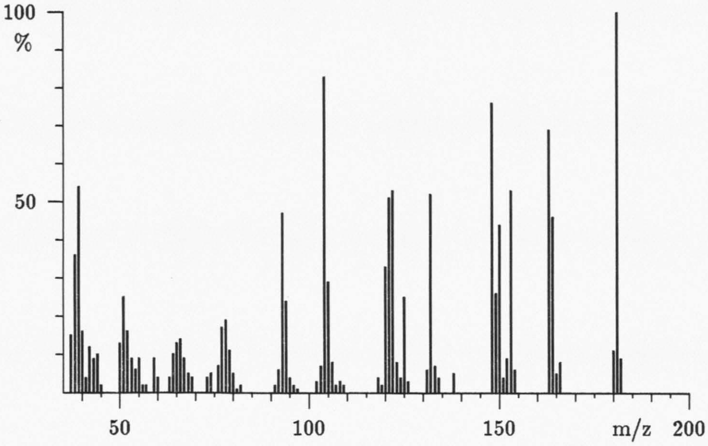


Fig. 1. 70 eV Mass spectrum of 7-carbmethoxy-1,2-dihydro-(3H)-pyrrolizin-1-ol (methyl hydroxydanaidoate).

insects. We have identified oxoisophorone (**I**) as one of the two main compounds along with traces of its hydrogenation product, (**II**). Recently, the same terpenoid cyclohexenone derivatives were found in another *Buddleja* species, *B. salvifolia* [16]. It is surprising to find (**I**) and (**II**) both as odour components in the secretions of flowers and insects, particularly since the butterflies (at least originally) are not sympatric with *Buddleja*. Oxoisophorone (**I**) is a widespread natural product of plants and is also known to be produced by microorganisms [17] along with the related cyclic ketones (**II**), (**V**) and (**VI**). (*E,E*)-α-Farnesene represents the second main component in the odour bouquet of *Buddleja,* which besides benzylalcohol and benzaldehyde contains the monoterpenes (*E*)- and (*Z*)-ocimene, α-pinene, geraniol and β-cyclocitral. (*E,E*)-α-Farnesene is also found in *A. echeria.*

The new bishomoterpenes (**X**) form another interesting group of compounds identified from the hairbrushes of *E. sylvester*. Similar compounds, (**XI**), identified by Meinwald *et al.* and by Edgar [18] from hairpencil extracts of *Danaus* spp. have been interpreted as fixatives for volatiles, particular important in promoting adherence of pheromone-transfer particles. While the structures of the *Danaus* compounds are related to geraniol, the new compounds **Xa–c** are obviously derived from linalool (**VII**), which is also present in the hairpencils of *E. sylvester* [13]. The biological function of **Xa–c** remains to be investigated. Other related compounds, showing a vinylcarbinol structure, are linalool (**VII**) and nerolidol found in *E. sylvester,* while the cyclic linalool derivatives (**VIII**) and (**IX**) are present in *A. echeria.*

The mass spectra of **Xb** and of linalool are given in Fig. 2. Similarities in the spectra are obvious; the *m/z* 71 fragment arises from the vinylcarbinol part of the molecules. The EI spectrum of **Xb** does not show the parent ion *m/z* 196 while ions *m/z* 178 (M-18) and *m/z* 160 (M-18-18) indicate two oxygen atoms in the molecule. The ion *m/z* 134 (M-44-18) suggests the presence of an aldehyde function. In the alcohol **Xa** the ion *m/z* 119 shifts to *m/z* 121. In the acid **Xc** the parent ion *m/z* 212 remains invisible, while the ions *m/z* 194 (M-18) and 134 (M-60-18), along with *m/z* 60 and *m/z* 73, indicate a carboxylic acid function. In the lower mass region of the compounds **X** the spectra look very similar.

Knowledge of the chemistry and biology of male pheromones of the Danainae [19] and Ithomiinae is limited, and the chemical data reported here contribute new information in this regard which may also be of interest from a taxonomic and phylogenetic point of view. The identification of danaidone (**XIII**) as a major component in the extracts of *E. sylvester* from Sri Lanka is remarkable, since *E. sylvester* from Australia was said to show only traces of danaidone while none was reported in a study of the subspecies from Taiwan (Formosa), although in both of these earlier studies hydroxydanaidal (**XIV**) was identified [13]. This apparent difference in the PA-derived hairpencil constituents of *E. sylvester* from different regions warrants confirmation since it could indicate a biochemical difference in the subspecies concerned

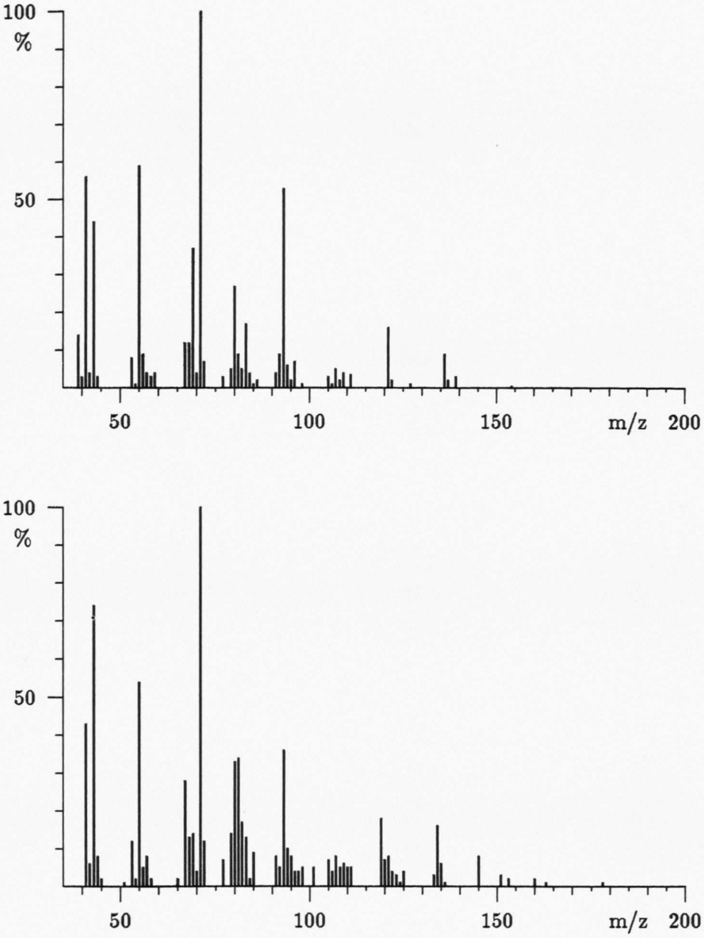


Fig. 2. 70 eV Mass spectra of linalool (above) and (*E*)-8-hydroxy-4,8-dimethyl-4,9-decadienal (below).

and be evidence of incipient or latent speciation. Alternatively, danaidone may not be a particularly important component in the complex hairpencil odour bouquet of *E. sylvester,* and the variability in its concentration may not have any influence on the ability of these subspecies to interbreed in nature. It should be noted that also with regard to other hairpencil constituents samples from Sri Lanka and Australia (possible subspecies) differed significantly.

The identification of hydroxydanaidal (**XIV**) on the hairpencils of *A. echeria* extends the number of danaine genera now known to secrete this widespread PA metabolite which is particularly prominent in species of the genus *Euploea* but is also found, usually with other dihydropyrrolizines, in *Tirumala, Parantica, Ideopsis* and *Danaus* [13]. Similarly, detection of the lactone **XII** in *P. hymenaea* increases the number of ithomiine genera (now five) known to secrete this PA metabolite [11], further indicating its wide, and this far unique, occurrence in the Ithomiinae.

The presence of the new dihydropyrrolizine, methylhydroxydanaidoate (**XV**), in three ithomiine species from different genera is of particular interest since, until now, dihydropyrrolizines have been found only in the subfamily Danainae, although Pliske has reported Ehrlich reagent-positive chemicals, which he believed to be dihydropyrrolizines, in ithomiine androconial extracts [3] and Brown has also suggested that dihydropyrrolizine metabolites of PAs are present in some ithomiine species [10].

With regard to the non-PA-derived compounds identified, it is tempting to speculate on their origin. As can be seen from the formula scheme, the compounds **I–IX** share a terpenoid carbon skeleton. Although it is known that some insects are able to synthesize terpenes *de novo* [20], it seems reasonable to suggest that, similar to other insects, the butterflies sequester these compounds or their precursors from plants. The cyclohexenones may well be derived from plant carotenoids [16], and aromatic compounds are also known to be widespread plant constituents.

Acknowledgements

We thank Dr. M. Boppré for helpful suggestions and for kindly providing extracts of *Amauris echeria*. Dr. T. E. Pliske provided Ithomiids from Venezuela. D. S. is grateful to Dr. J. R. Morello, President of the Argentine Nat. Park System, for permission to work in Iguazú. Financial support by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

[1] M. Boppré, in: Symp. R. ent. Soc. **11,** 259 (= The Biology of Butterflies, R. I. Vane-Wright and P. R. Ackery, eds., Academic Press, London); P. R. Ackery and R. I. Vane-Wright, Milkweed Butterflies: Their Cladistics and Biology, British Museum (Natural History) (1984).
[2] W. A. Haber, Ph.D. Thesis, Univ. of Minnesota (1978); see also [1].
[3] T. E. Pliske, Ann. Ent. Soc. Am. **68,** 935 (1975).
[4] W. Boland, P. Ney, L. Jaenicke, and G. Gassmann, in: Analysis of Volatiles (P. Schreier, ed.), p. 371, W. de Gruyter & Co., Berlin 1984.
[5] V. J. Freer and P. Yates, Chem. Let. **1984,** 2031.
[6] J. N. Marx and F. Sondheimer, Tetrahedron Suppl. **8,** 1 (1966).
[7] J. E. Cane, R. Iyengar, and M.-S. Shiao, J. Am. Chem. Soc. **103,** 914 (1981).
[8] E. J. Corey, N. W. Gilman, and B. E. Ganem, J. Amer. Chem. Soc. **90,** 5616 (1968).
[9] M. Boppré, Naturwissenschaften **73,** 17 (1986).
[10] K. S. Brown, Rev. Brasil. Bio. **44,** 435 (1984).
[11] J. A. Edgar, C. C. J. Culvenor, and T. E. Pliske, J. Chem. Ecol. **2,** 263 (1976).
[12] J. Meinwald, C. J. Boriack, D. Schneider, M. Boppré, W. F. Wood, and T. Eisner, Experientia **30,** 721 (1974).
[13] J. A. Edgar, C. C. J. Culvenor, and L. W. Smith, Experientia **27,** 761 (1971); H. Komae, A. Nishi, T. Tanaka, N. Hayashi, C. Wesou, and Y. Kuwahara, Biochem. Sys. Ecol. **10,** 181 (1982); J. A. Edgar, J. Zool. (Lond.) **196,** 385 (1982).
[14] T. Eisner, L. B. Hendry, D. B. Peakall, and J. Meinwald, Science **172,** 277 (1971).
[15] G. Birgersson, F. Schlyter, J. Löfquist, and G. Bergström, J. Chem. Ecol. **10,** 1069 (1984).
[16] R. Tabacchi, J. Garnero, Ch. Quellet, and G. Tsoupras presented at the 10th Intern. Congress of Essential Oils, Fragrances and Flavors; Washington D.C. November 16–20, 1986.
[17] Y. Mikami, M. Arita, Y. Obi, and T. Kisaki, Agric. Biol. Chem. **45,** 791 (1981); N. Hori, T. Hieda, and Y. Mikami, Agric. Biol. Chem. **48,** 213 (1984).
[18] J. Meinwald, Y. Meinwald, and P. H. Mazzochi, Science **164,** 1174 (1969); J. Meinwald, W. R. Thompson, T. Eisner, and D. F. Owen, Tetr. Let. **1971,** 3485; J. Meinwald, A. M. Chalmers, and T. E. Pliske, J. Chem. Soc., Chem. Commun. **1969,** 86; J. Meinwald, A. M. Chalmers, T. E. Pliske, and T. Eisner, Tetr. Let. **1968,** 4893.
[19] D. Schneider presented at the 2nd Intern. Monarch Conference; Los Angeles September 1986.
[20] G. M. Happ and J. Meinwald, Advan. Chem. Ser. **53,** 27 (1966); J. Meinwald, G. M. Happ, J. Labows, and T. Eisner, Science **151,** 79 (1966).