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# DISCOVERY AND IDENTIFICATION OF AN ANTIJUVENILE HORMONE FROM *CHRYSANTHEMUM CORONARIUM*

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Natural products chemistry has served as an important resource of biologically active chemicals useful in medicine and plant protection. Historically, the early insecticides were derived directly as plant extractives such as nicotine, nornicotine, pyrethrins, rotenone etc. More recently insect growth regulators, isolated from animals and plants, (Bowers, 1985) have supplied the prototypic chemistry that led to the development of the first biorational insect control agents. Synthetic optimization of the insect juvenile hormone has yielded unique, intrinsically non-toxic products for insect control. These hormonally based products inhibit normal metamorphosis preventing adult development and reproduction. This mode of action is especially appropriate for the control of insects in which the adult stage is of economic importance, i.e., mosquitoes, flies and fleas. Insect pests of plants, on the other hand, produce their damage during the immature, feeding stages which cannot be interrupted by treatment with the juvenile hormone based growth regulators. A more satisfactory approach for the control of immature insects requires limitation of the duration of their feeding periods. Classical research in insect endocrinology has shown that ablation of the gland responsible for the production of the juvenile hormone (i.e., the corpus allatum=CA) initiates a telescoped developmental period including precocious metamorphosis into diminutive sterile adults. A chemical method simulating surgical ablation would be a discrete, insect-specific method of insect control particularly appropriate to plant protection.

Since plants had been shown to possess secondary compounds with juvenile hormone activity we antecipated that they might have alternative defensive strategies based upon antihormones. A search among plants revealed two naturally occuring chromenes with antijuvenile hormone activity in the plant *Ageratum houstonianum*, a member of the plant family Compositae (Bowers, 1976; Bowers et al., 1976). Because of their ability to induce precocious metamorphosis and their chromene structure we called the antihormonal compounds precocene I and II.

From studies of precocene metabolism (Soderlund et al., 1980), pharmacokinetics (Haunerland & Bowers, 1985), reaction with model substrates, (Aizawa et al., 1985) and cytological investigations (Unnithan et al., 1977), it appears that the precocenes are activated by oxidation to form highly reactive epoxides that destroy the parenchymal cells of the CA by nucleophilic alkylation. The resultant loss of JH secretion induces several disruptive biological actions including the induction of precocious metamorphosis, sterilization, diapause induction, elimination of sex pheromone production and antifeeding. Extensive research has indicated that paurometabolous species are more susceptible to the precocenes than the holometabola because of a combination of favorable distribution mechanisms and limited metabolism (Haunerland & Bowers, 1985).

Continued efforts to find additional antihormonal models in plants has now revealed a new compound of novel chemistry possessing significant antijuvenile activity. The possible presence of antijuvenile hormone activity in *Chrysanthemum coronarium* was suspected from the preliminary bioassay of fractions of a dicholoromethane extract from a European population of *C. coronarium* received in our laboratory, however the limited sample size precluded successful isolation of the active principle(s).

Although of Old World origin, *C. coronarium* exists widely as an introduced ornamental and thrives as a wild annual in many areas of coastal California and Mexico. An abundant sample of *Chrysanthemum coronarium* was collected from Southern California (Newport Beach) and subjected to chemical and biological studies. Voucher specimens are on file at the University of California Herbarium in Irvine.

## MATERIALS AND METHODS

*Chrysanthemum coronarium* from California

was air dried and ground in a Wiley Mill prior to solvent extraction. The dried leaves and twigs were extracted with chloroform overnight at room temperature, filtered, and the solvent removed under vacuum. The dried extractives were dissolved in methanol and the insoluble residue was removed by filtration. The methanol soluble material was reduced in volume under vacuum and chromatographed over Florisil (100-200 mesh, deactivated with 7% $H_2O$). Elution of the column was performed in gradient fashion from 100% hexane to 100% methanol. Column development was continuously monitored by long wave UV light, and substantive fractions were monitored by thin-layer chromatography on silica gel plates. From approximately 200 fractions the elution profile indicated four major constituents. Compound A and then compound B eluted first, whereas compounds C and D eluted subsequently as an unresolved mixture. The latter were separated by permeation chromatography on Sephadex LH-20 packed and developed in methanol.

Chemical structures were determined *via* a combination of mass spectra, $^{1}H$ and $^{13}C$ nuclear magnetic resonance, infrared and ultraviolet spectrometric methods.

Crude extracts and pure compounds were evaluated for toxicity and antijuvenile hormone activity (Bowers, 1976) within a range of concentrations from 78 $\mu g/cm^2$ to 0.7 $\mu g/cm^2$ on *Oncopeltus fasciatus*, in contact residue experiments.

## RESULTS

Fig. 1 indicates the successful fractionation procedure yielding four major compounds by combination of adsorption chromatography on Florisil (gradient elution from hexane to methanol) and permeation chromatography on Sephadex LH-20 (in methanol).

Biological assays indicated significant insecticidal activity in a number of fractions, however, anti-juvenile hormone activity was not expressed until the pure major constituents were tested individually.

The polyacetylenic nature of the isolated compounds was suggested from the $^{13}C$-NMR spectra of compound B (the major component of the 4 isolated compounds) which revealed a peak at 4.6 ppm indicative of a methyl group attached to a triple bond (i.e., $CH_3$-C≡C-). The constituents (compounds A,B,C), examined by ultraviolet, infrared and $^{1}H$-NMR were found

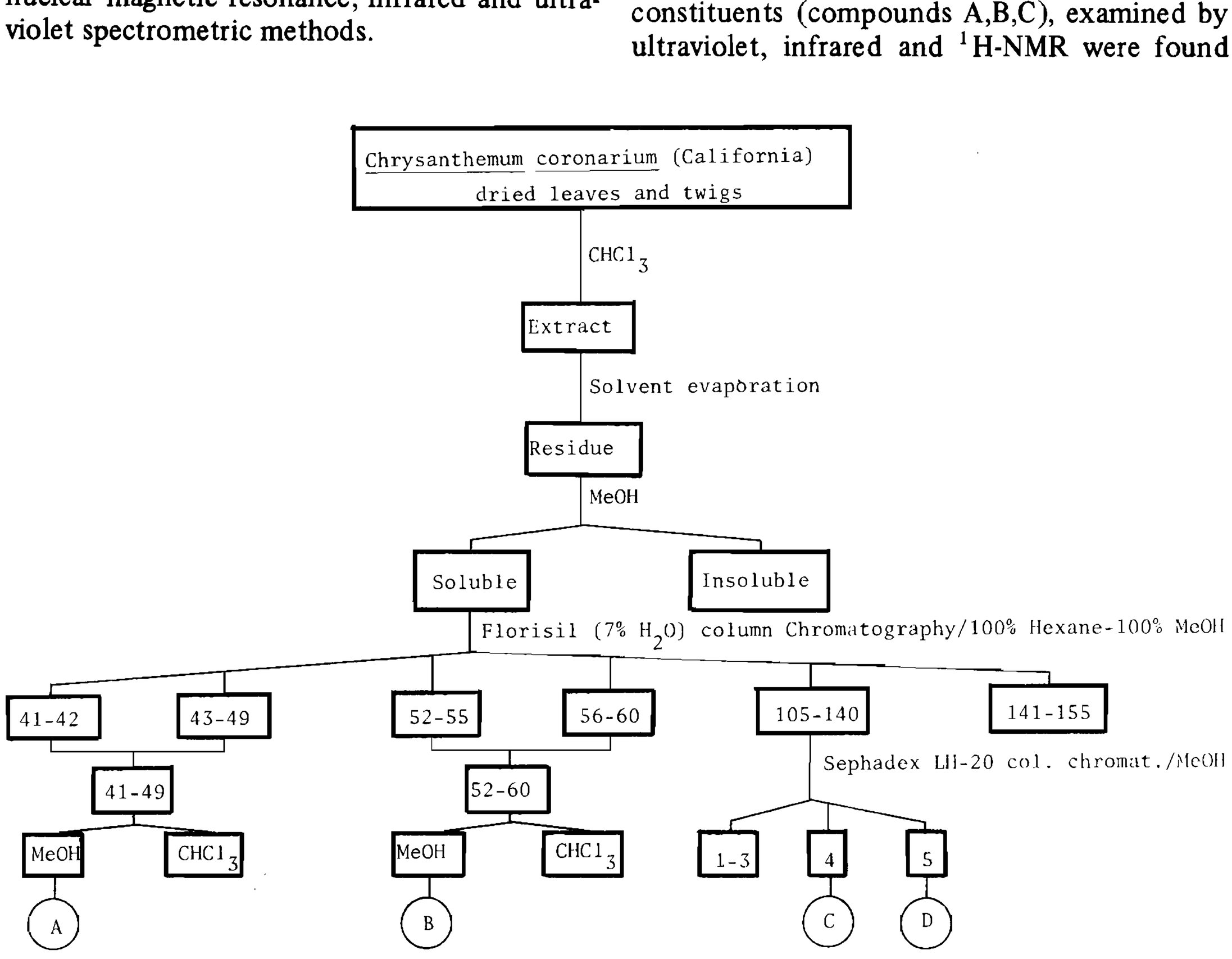


**Fig. 1: Fractionation procedure for the isolation of the polyacetylenes A-D.**

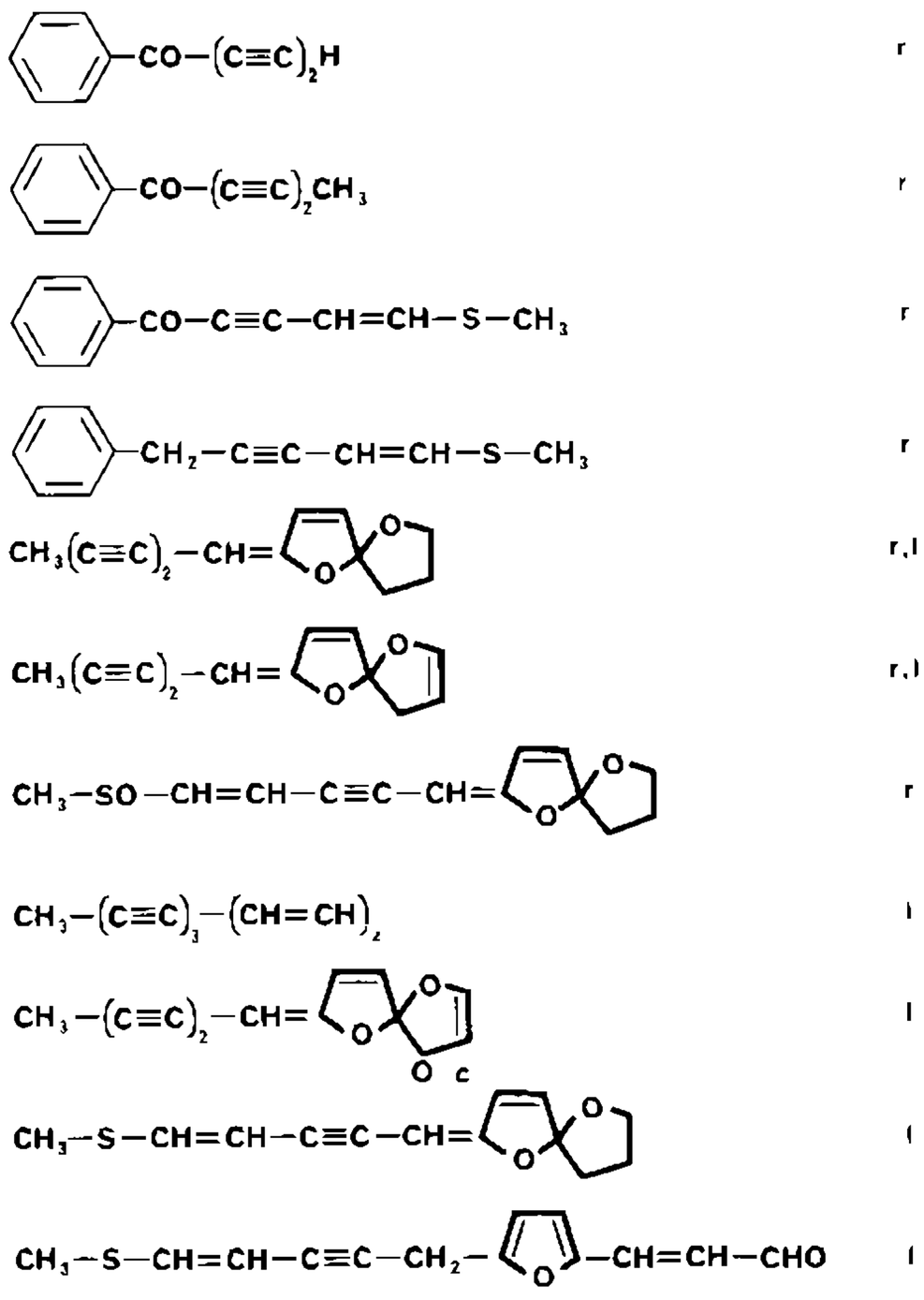


Fig. 2: Polyacetylene chemistry of *Chysanthemum coronatiom* Compositae).

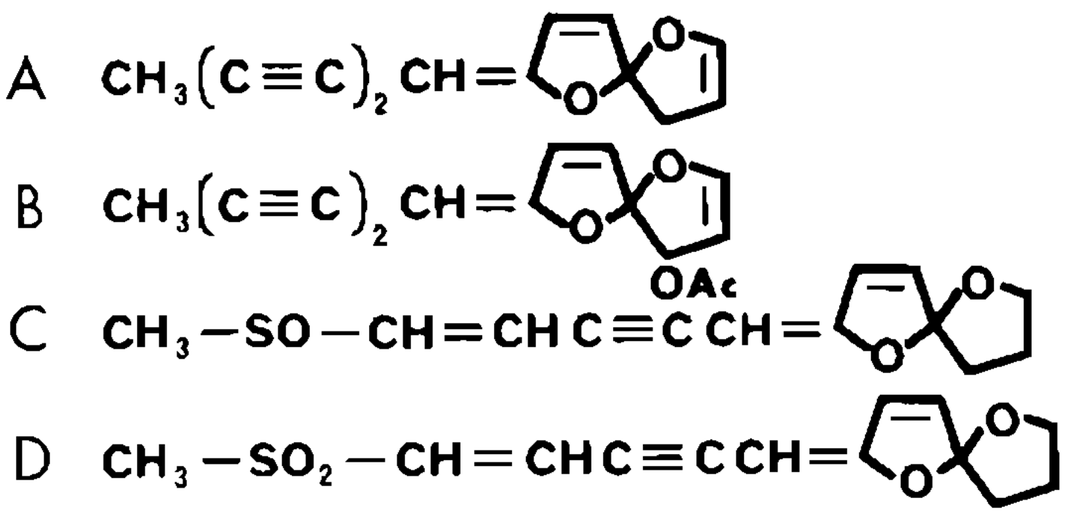

Fig. 3: Major biologically active polyacetylenes of North American *Chrysanthemum coronarium* (Compositae).

to be identical with several polyacetylenes identified earlier by Bohlmann (1964) in *C. coronarium* (Fig. 2). Fig. 3 indicates the four major polyacetylenic compounds isolated from the California sample of *C. coronarium.* Compound D, (i.e., the sulfone) although not previously reported to occur naturally, has been synthesized from the sulfide (Bohlmann, 1964). While the stereochemistry of D has not been unambiguously elucidated it appears that compound D in our sample is the *trans-cis* stereoisomer.

The biological studies, recorded in Table, indicate that compound B is the most toxic, whereas the toxicity of compound D is negligible. While compound C appears toxic at

TABLE

Biological evaluation of polyacetylenes isolated from *C. coronarium* on second stage nymphs of *Oncopeltus fasciatus*

| | % Mortality % Precocious adults | | | |
|---|---|---|---|---|
| | Concentration ($\mu g/cm^2$) | | | |
| Compound | 0.78 | 3.9 | 7.8 | 19.5 |
| A | 0 | 25 | 100 | 100 |
| B | 10 | 100 | 100 | 100 |
| C | 0 | 0 | 0/10 | 50/50 |
| D | 0 | 0 | 0 | 0 |

high concentrations it possesses antijuvenile hormone activity at 19 $\mu g/cm^2$, and causes approximately 50% of treated 4th stage milkweed bug nymphs to develop directly into precocious, sterile adults. Since the polyacetylenes tend to polymerize on exposure to light, all tests were conducted in the dark. This mode of evaluation was necessary to obtain the antijuvenile hormonal activity.

## DISCUSSION

Chemical characterization studies confirm the structure of the antihormone as the polyacetylenic sulfoxide, compound C. Early recognition of its photolability was an important criterion in relocating the test specimens during the antijuvenile hormone assay to preclude exposure to light. Although the polyacetylenes as a class of natural products have been recognized to possess a variety of biological activities (Towers & Wat, 1978), the present study is the first to reveal an antihormonal action. The full spectrum of biological activity against insects remains to be determined as does the specific mechanism by which the antijuvenile hormonal response is effected. As with the precocenes we have undertaken metabolism, pharmacokinetic, and histological studies to resolve the nature of the biological activity of this unique compound.

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