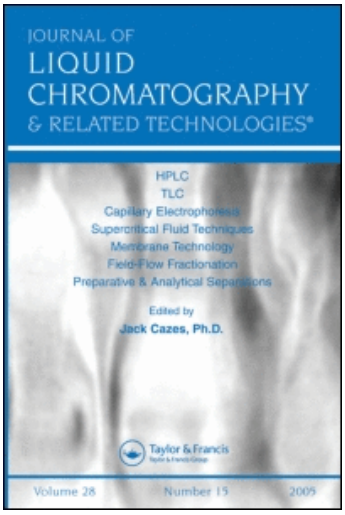

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Analysis of Insect Hormones by Means of a Radial Compression Separation System

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ANALYSIS OF INSECT HORMONES BY MEANS OF A RADIAL COMPRESSION SEPARATION SYSTEM

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ABSTRACT

The use of a radial compression separation system for the analysis of insect hormones is described. By simple isocratic elution, both steroid molting hormones and terpenoid juvenile hormones are rapidly separated. The system is used to analyze the metabolism of juvenile hormone by an established cell line of Drosophila melanogaster.

INTRODUCTION

Two major groups of non-peptide hormones influence the development of insects: ecdysteroids and juvenile hormones (JH) (Fig. 1). The ecdysteroids, polyhydroxy steroids that initiate molting, are present in both insects and crustaceans (1,2). The juvenile hormones are homosequiterpenoids that mediate the qualitative nature of the molting process. Juvenile hormones have been found conclusively so far only in insects. The separation techniques for these two groups of hormones include low-pressure column, thin-layer (TLC), gas-liquid (GLC), and high-performance

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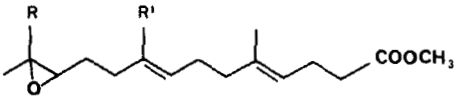
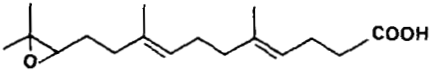


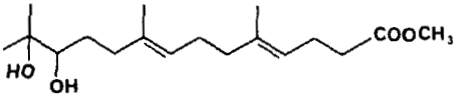
JH I: R: Et, R': Et

JH II: R: Et, R': Me

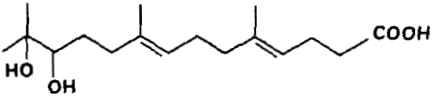
JH III: R: Me, R': Me



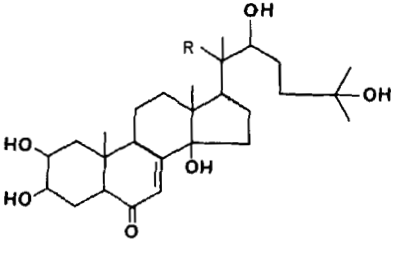
JH III acid



JH III diol



JH III acid–diol



ecdysone: R: H

20-hydroxyecdysone: R: OH

FIGURE 1. Chemical structures of the insect hormones described in the text.

liquid chromatography (HPLC) (3,4). Radially compressed columns enable even more rapid separations of these compounds than have previously been reported.

MATERIALS AND METHODS

The juvenile hormones (JH I, methyl (2E,6E,10Z)-(10R,11S)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate; JH II, methyl (2E,6E,10Z)-(10R,11S)-10,11-epoxy-3,7,11-trimethyl-2,6-tridecadienoate; and JH III, methyl (2E,6E)-10-11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate) were obtained from Calbiochem. The metabolites of JH III (the JH III acid, JH III diol, and JH III acid-diol) were prepared according to previously published procedures (5,6). Radiolabeled JH III (10-^{3}H) was obtained from New England Nuclear (sp. act. 11 Ci/mmol). Ecdysone (2β,3β,14α,22R, 25-pentahydroxy-5β-cholest-7-en-6-one) was obtained from Simes and 20-hydroxyecdysone (2β,3β,14α,20R,22R, 25-hexahydroxy-5β-cholest-7-en-6-one) from Rohto Pharmaceutical Co. Hormones and metabolites were purified by TLC or HPLC if necessary.

Analyses were performed with a Waters system, consisting of a M-45 solvent delivery system, U6K injector, 440 UV detector set to 254 nm, RCM-100 radial compression module, and a C_8 Radial-Pak cartridge (0.5 i.d. x 10 cm). Data were analyzed with a reporting integrator (3390A, Hewlett-Packard). The solvents, reagent grade water (Milli-Q, Millipore Corp.) and methanol (Nanograde, Mallinkrodt), were filtered and degassed by means of extensive stirring prior to use. The ecdysteroids were eluted with 50% methanol at a flow rate of 1.0 ml per min, the JH homologs with 75% methanol at 2.0 ml per min, and the JH metabolites with 60% methanol at 1.5 ml per min.

The Drosophila melanogaster Kc cells were cultured as described previously (7). To examine the metabolism of ^{3}H-JH III by the Kc cells, approximately 10^{8} cells were washed in TMK buffer

(10 mM Tris, 5 mM $MgCl_2$, 150 mM KCl, pH 7.4) and resuspended in 1 ml of TMK buffer, containing 1 µCi of the labeled hormone. The cells were incubated at 25° for 30 min with gentle agitation. The cells were then pelleted and the supernatant was extracted with 3 x 2 ml ethyl acetate. The organic phases were combined and concentrated. The residue was resuspended in 1 ml of methanol, filtered (0.22 µm, Fluoropore membrane, Millipore Corp.) and concentrated to 50 µl prior to injection.

RESULTS

Figure 2 is a chromatogram of ecdysone and 20-hydroxyecdysone. Preliminary data indicate that other ecdysteroids can also be separated by this system.

The separation of the three principal homologs of JH is shown in Figure 3. Reduction in the side-chain length from ethyl to methyl results in an increase in the polarity of the molecule, reflected in a decreased elution volume.

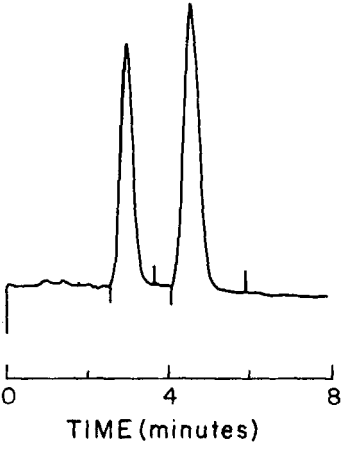


FIGURE 2. Chromatogram of 20-hydroxyecdysone (2.68 min, 88 pmol) and ecdysone (4.23 min, 138 pmol). Solvent, 50% aq. methanol; flow-rate, 1.0 ml/min.

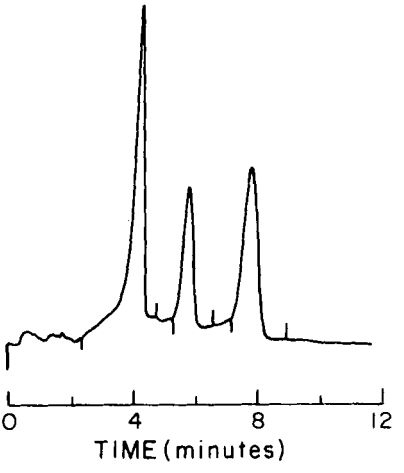


FIGURE 3. Chromatogram of JH III (3.90 min, 1.0 nmol), JH II (5.44 min, 0.4 nmol), and JH I (7.38 min, 0.7 nmol). Solvent, 75% aq. methanol; flow-rate, 2 ml/min.

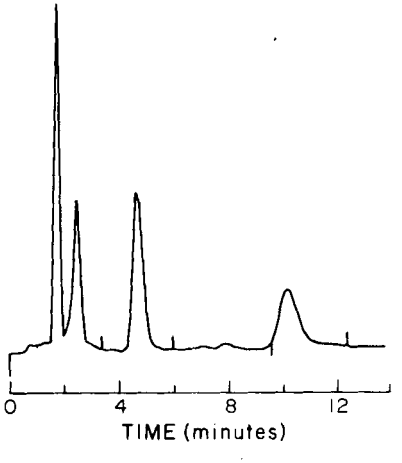


FIGURE 4. Chromatogram of JH metabolites: JH III acid-diol (1.56 min, 0.46 nmol), JH III acid (2.27 min, 0.33 nmol), JH III diol (4.41 min, 0.48 nmol), and JH III (9.60 min, 0.37 nmol). Solvent, 60% aq. methanol; flow-rate, 1.5 ml/min.

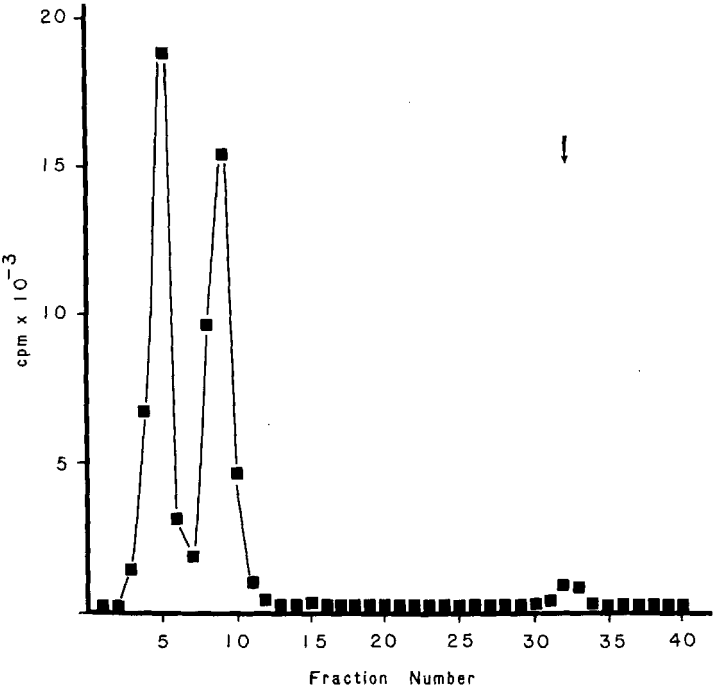


FIGURE 5. Radiochromatogram following HPLC analysis of ^{3}H-JH III, incubated with Drosophila Kc cells. Solvent, 60% aq. methanol; flow-rate, 1.5 ml/min, fractions, 0.45 ml/0.3 min. Arrow, elution of authentic JH III.

Figure 4 is a chromatogram of the predominant biological inactivation products of JH. The JH acid is formed in biological systems by the action of an esterase, the JH diol by hydrolysis of the diol by an epoxide hydrolase, and the JH acid-diol by the sequential action of both of these two enzymes. Depending on the enzyme system, either the acid or the diol may be the preferred intermediate in the acid-diol formation.

In order to determine whether the above separation system could be used to analyze metabolites from a biological system, ^{3}H-JH III was added to an established cell line of Drosophila

melanogaster. These cells have especially high levels of the catabolic enzymes (8). As seen in Fig. 5, almost all of the radiolabeled hormone was metabolized to the acid and the acid-diol. By addition of an internal JH III standard, it was ascertained that the biological extract did not alter the elution volume of the authentic hormone.

DISCUSSION

Because the two groups of insect developmental hormones, ecdysteroids and juvenile hormones, interact in a complex manner (1), it is important to consider the effects and titers of both classes of hormones in insect systems. HPLC has been used for such studies. Lafont et al. (9) have applied reversed-phase HPLC for the separation of complex mixtures of ecdysteroids and their metabolites. The conditions reported in the present paper, though perhaps not as well suited for complex mixtures of ecdysteroids, provide a more rapid method for the analysis of ecdysone and 20-hydroxyecdysone, the two molting hormones most frequently encountered in biological systems (10,11). This separation system should be suitable for the routine determination of the ecdysone to 20-hydroxyecdysone ratios in arthropod blood (12).

Rapid separations by reversed-phase HPLC have also been developed for JH (13), primarily as a purification step prior to mass spectrometry. The advantages of the conditions reported in the present publication are speed, use of the same solvents for all separations, and relatively low cost of the radially-compressed columns.

Although more extensive purification of biological samples is required for direct quantitation of these hormones by UV absorption, the separations described are adequate for hormone analysis by an indirect method, such as radioimmunoassay (14,15) or scintillation spectrometry. The separation of radiolabeled JH

metabolites described in this report indicates that no additional purification is necessary for HPLC analysis following extraction.

ACKNOWLEDGMENTS

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