Short Communication

Efficient Isolation of a Large Amount of 20-Hydroxyecdysone from *Vitex madiensis* (Verbenaceae) by Droplet Counter-Current Chromatography

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Received January 17, 1984

In our continuing search for alternative insect control agents based on natural products, we examined the root-bark of the West African medicinal plant *Vitex madiensis* (Verbenaceae) which is known to be relatively free from insect attack. The chemical constituent of this plant has not been reported so far. When the crude methanol extract of the fresh root-bark of *V. madiensis* was incorporated into artificial diets^{1,2)} optimized for two economically important agricultural pest insects, the pink bollworm, *Pectinophora gossypiella* and the fall armyworm, *Spodoptera frugiperda*, they failed to undergo normal molting behavior. Death resulted from the disruption of the molting cycle.³⁾ Apparently, the *V. madiensis* extract disrupted the molting cycle by causing each new cuticle to harden in an untimely manner and the outcome was a larva with up to three cuticular head capsules all superimposed on each other. This type of reaction (inhibition of insect ecdysis) suggested that phytoecdysteroids were responsible for the activity of the extract.⁴⁾ In fact, when the extract was separated into its ether, ethyl acetate and water-soluble portions, the extract with ethyl acetate, which is usually used to extract phytoecdysteroids, contained the active fraction. HPLC fractions of this ethyl acetate extract resulted in the isolation of two bioac-

tive compounds $C_{27}H_{44}O_7$, mp 241~242°C and $C_{27}H_{44}O_7$, noncrystalline. Spectroscopic identification (UV, IR, MS, 1H -NMR and ^{13}C -NMR) showed the presence of 20-hydroxyecdysone and ajugasterone C, respectively.⁵⁾ This was confirmed by direct comparison with the authentic samples previously isolated from the *Ajuga* plants.⁶⁾

For a detailed biochemical study, sufficient quantities of the phytoecdysteroids are needed. Recently, we reported that droplet counter-current chromatography (DCCC) (Model DCC-300-G2, Tokyo Rikakikai Co., Tokyo, Japan) was an especially efficient preparative method for the separation of phytoec-

FIG. 1. DCCC of the Ethyl Acetate Extract of *V. madiensis* (2.65 g) with $CHCl_3$ -MeOH- H_2O (13:7:4, v/v), by the Ascending Method; 1.5 ml per Fraction.

FIG. 2. RLCC of the Ethyl Acetate Extract of *V. madiensis* (2.17 g) with $CHCl_3$ -MeOH- H_2O (13:7:4, v/v) by the Ascending Method; 1.4 ml per Fraction.

dysteroids. Hence, DCCC was employed for the isolation of adequate amounts of the *V. madiensis* phytoecdysteroids. The ascending method was utilized in our DCCC separation with chloroform-methanol-water (13:7:4, v/v). We fractionated 2.65 g samples of the ethyl acetate extract and collected 1.5 ml per fraction. Figure 1 provides that weights of the elute vs. the fraction number, and shows a good baseline separation. Even though DCCC requires only small volumes of solvent, more than 1 g of 20-hydroxyecdysone was rapidly and nondestructively isolated and fully recovered from a 2.65 g injection.

An alternative counter-current chromatography, the rotation locular counter-current chromatograph (RLCC) (Model RLCC-A, Tokyo Rikakikai Co., Tokyo, Japan), was also investigated by using the same solvent system as for DCCC. However, this method could not separate 20-hydroxyecdysone and ajugasterone C, both of which gave the same *R_f* value on a TLC plate using an organic layer of chloroform-methanol-water (13:7:4, v/v), in Fig. 2. The root-bark of fresh *V. madiensis*

contained 0.4% 20-hydroxyecdysone and 0.2% ajugasterone C (3.6% and 1.4% from the methanol extract, respectively). To our knowledge, there are, so far, no other higher plants which contain greater quantities of phytoecdysteroids than *V. madiensis*.

Acknowledgments. Insects were kindly supplied by the agencies of the USDA in Tifton, GA, Phoenix, AZ, and Brownsville, TX. We are grateful to Takasago Perfumery Company for the purchase of the DCC-300-G2 instrument. The authors thank Professor Y. Tanada for reading the paper.

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