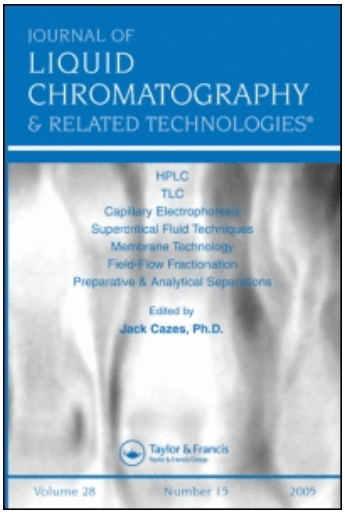

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EFFICIENT ISOLATION OF THE INSECT GROWTH INHIBITORY FLAVONE GLYCOSIDE RUTIN FROM TWO TROPICAL MEDICINAL PLANTS BY ROTATION LOCULAR COUNTERCURRENT CHROMATOGRAPHY (RLCC)

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ABSTRACT

An insect growth inhibitory flavone glycoside, rutin, was efficiently isolated from two tropical medicinal plants Solanum incanum and Esenbeckia pumila by rotation locular countercurrent chromatography (RLCC).

INTRODUCTION

Flavonoids are a widely distributed group of secondary metabolites, universally present in vascular plants, and responsible for much of the natural coloring in nature. They are also important for normal plant growth and development, and for defense against insect and microbial attack. Flavonoids

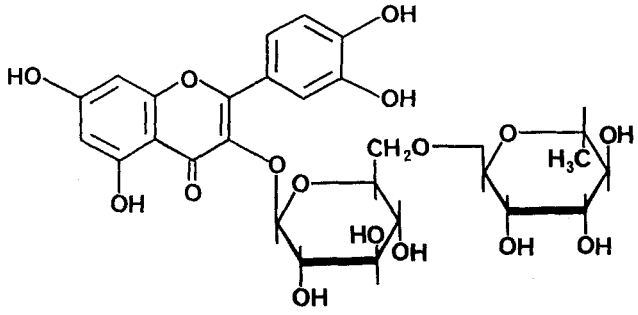
constitute the active ingredients in many medicinal plants. They have been found to possess various pharmaceutical activities both in vitro and in vivo such as antiinflamatory, antiplatelet aggregation, antimicrobial, antiviral, and antihypertension.

In our continuing study of biologically active natural products, countercurrent chromatographic techniques have been demonstrated to be useful, especially in the isolation of polar compounds. Countercurrent chromatographies, droplet countercurrent chromatography (DCCC) and rotation locular countercurrent chromatography (RLCC), have an inherently large sample capacity, no adsorbent, typically a low solvent consumption. We have previously reported our own examples of the isolation of various phytochemicals by DCCC and RLCC including the general principles (1,2). This paper is limited to a few recent examples of the isolation of a flavone glycoside by RLCC. We describe an efficient and simple method for the isolation of an insect growth inhibitory flavone glycoside, rutin, from two tropical medicinal plants Solanum incanum (Solanaceae) and Esenbeckia pumila (Rutaceae).

RESULTS AND DISCUSSIONS

In our preliminary screening, the crude methanol extracts of the leaves of two tropical medicinal plants, S. incanum and E. pumila, exhibited insect growth inhibitory activity by an artificial diet feeding assay using important pest insects, Heliothis virescens (tobacco budworm) and Pectinophora gossypiella (pink bollworm) (3). Bioassay guided fractionation led to the isolation of the same active principle from both S. incanum and E. pumila and identified the flavone glycoside, rutin, by means of spectroscopic data. In these studies, rutin

was efficiently isolated from rather crude extracts at a semipreparative scale by RLCC.



Rutin is one of the most common polar flavonoid glycosides in many plants, and recently, it has been found to be toxic to many insects, including H. virescens and P. gossypiella as well as H. zea (corn earworm) (4,5).

S. incanum, in general common everywhere in the tropics, especially along roadsides, is considered to be relatively immune to insect attack. At least some of this immunity may be attributed to steroid glycoalkaloids, compounds with insecticidal activity which occur predominantly in the Solanaceae. In addition to the steroid glycoalkaloids (6), a non-toxic growth inhibitory flavone glycoside, rutin, was also isolated from S. incanum. Incorporation of rutin as high as 5% in the artificial diet resulted in abnormally small, but live, larvae. The non-toxic growth inhibitory activity might be due to some effect on larval metabolism, especially since rutin evoked a feeding deterrence response in a leaf disk assay. The apparent diversity of protective strategies included in the chemical defense of S. incanum is especially essential because of wide differences in the susceptibility of insect species to any one defense. Thus,

the use of more than one chemical in the defense strategy confers a potential for resistance in S. incanum foliage.

Similarly, the Rutaceae is rich in limonoids, coumarins and alkaloids which are usually toxic to insects. In fact, some quinolinone alkaloids were identified in an Esenbeckia species (7).

The isolation of rutin has been reported from many plants by various methods. Previously, preparative TLC with SiO_2 and cellulose, and column chromatography on SiO_2, sephadex LH-20 and polyamide have been used for the isolation of rutin (8-11). However, these methods mse solid packing materials which often cause the irreversible absorption of polar compounds such as rutin. In addition, all of these methods except TLC are time consuming. In contrast, countercurrent chromatographic techniques such as DCCC and RLCC, without solid packing materials, are useful for the isolation of polar compounds such as rutin and other flavonoid glycosides as well (12,13). These liquid-liquid chromatographic techniques may also avoid decomposition of unstable polar compounds. An efficient and simple method for the isolation of rutin by RLCC using only a gradient elution as the moving phase has been established.

When fractionation is followed by bioassay to isolate active principles from crude extracts, the first step is usually to partition between water and organic solvents such as n-hexane, ether (or methylene chloride), ethyl acetate, and n-butanol, in that order. If the biological activity is found in polar fractions such as the ethyl acetate and/or n-butanol fraction, countercurrent chromatographies may be considered practical. Many flavonoids are polar but still extractable with organic solvents. Therefore, the RLCC technique may be useful in their

purification. Incidentally, some polar compounds are not separated by countercurrent chromatography. For example, fairly large amounts of tannin, which are known to be toxic to insects (14), were detected by TLC in the crude extracts of S. incanum and E. pumila. In spite of many attempts, they have not yet been completely separated.

EXPERIMENTAL

Apparatus. The present study was carried out with RLCC apparatus (Tokyo Rikakikai, Tokyo, Japan). UV spectra were recorded on a Hitachi 100-80 spectrometer. All NMR spectra acquired on a JEOL GX 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) with signals reported in ppm from internal TMS. FAB-MS spectra were acquired on a JEOL DX 303 HF.

(1) Rutin from S. incanum. The methanol extract (40 g) of the leaves of S. incanum collected near Nairobi and Mombasa in Kenya was suspended in water and a small amount of methanol. The water suspension was extracted with n-hexane and chloroform to remove non-polar colored components. The water layer was filtered to remove precipitated material (28 g) and injected directly to the sample roup of the RLCC apparatus. Water was used as the stationary phase and the columns were filled with water before the sample was injected. A gradient elution with water saturated n-hexane-ether (1 l), water saturated ethyl acetate (1.6 l) and the upper layer of ethyl acetate-propanol-water 10:1:2 (1.3 l) was used in an ascending method. The flow rate of eluent was 1-2 ml/min., and the experiment was monitored by SiO_2-TLC (chloroform-methanol-water 7:3:1, organic layer) with vanillin sulfuric acid and ferric chloride spray reagents. The

final solvent system, the upper layer of ethyl acetate-propanol-water 4:1:1, afforded yellow crystals of rutin (30 mg), followed by crystallization with a mixture of methanol and water.

(2) Rutin from E. pumila. The dried powdered leaves of E. pumila (0.6 kg), collected near Sào Paulo, were extracted with dichloromethane and methanol at room temperature for 10 days. The methanol extract was evaporated to dryness at 40°C to give a dark brown residue (15 g). Part of this residue (2.0 g) was suspended in a mixture of water and a small amount of methanol. The water suspension was partitioned with chloroform to remove non-polar components. The water layer was concentrated in vacuo to give a syrup (400 mg), which was dissolved in 20 ml of water. The water solution was injected into the RLCC sample roup after filtration. A stationary phase of water and a moving phase of water saturated ether (500 ml), water saturated ethyl acetate (500 ml) and the upper layer of ethyl acetate-propanol-water (10:1:2 and 4:1:1, 1 l) proved to be a successful solvent system in the ascending method. The flow rate was 1 ml/min., and the eluent was collected in 25 ml fractions. Each fraction was checked by SiO_2-TLC ($CHCl_3$-MeOH 8:2) with vanillin sulfuric acid spray reagent. Fractions 37-51 gave yellow crystalline rutin (30 mg), which was then crystallized with a mixture of methanol and water.

Rutin FAB-MS ($M^+ - H$): 609. λ_{max} (MeOH): 355, 255; λ_{max} (MeOH + NaOH): 405, 325, 264. ^1H NMR (500 MHz, CD_3OD): 7.65 (1H, d, J=2, H-2'); 7.62 (1H, dd, J=8.5, 2, H-6'); 6.87 (1H, d, J=8.5, H-5'); 6.39 (1H, d, J=2, H-6); 6.21 (1H, d, J=3, H-8);

5.10 (1H, d, J=7.3, H-1 glucose); 4.52 (1H, d, J=1.7, H-1 rhamnose); 1.1 (3H, d, J=6.4, Me-rhamnose).

^{13}C NMR (125 MHz, CD_3OD): 179.4 (C-4); 166.0 (C-7); 163.0 (C-5); 159.3 (C-9); 158.3 (C-2); 149.8 (C-4'); 145.8 (C-3'); 123.5 (C-6'); 123.2 (C-1'); 117.7 (C-5'); 116.0 (C-2'); 104.7 (C-10); 99.9 (C-6); 94.8 (C-8); 105.6 (C-1'); 75.7 (C-2'); 78.2 (C-3'); 71.4 (C-4'); 77.2 (C-5'); 68.5 (C-6') [Glucose]; 102.4 (C-1''); 72.1 (C-2''); 72.3 (C-3''); 73.9 (C-4''); 69.7 (C-5''); 17.8 (C-6'') [Rhamnose].

Bioassay. The artificial diet feeding assay was performed according to the method described by Chan et al using P. gossypiella and H. virescens (3).

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