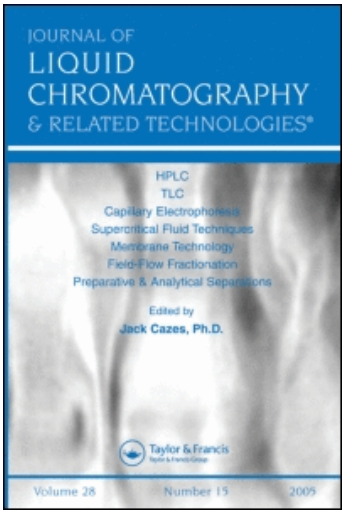

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PREPARATIVE REVERSED-PHASE LIQUID CHROMATOGRAPHIC ISOLATION OF AZADIRACHTIN FROM NEEM KERNELS (1)

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ABSTRACT

Azadirachtin is a tetranortriterpenoid insect feeding deterrent and growth regulator present in the kernels of the neem tree, Azadirachta indica A. Juss. A procedure was developed whereby 8.7 g of >90% pure azadirachtin was obtained from 48.2 kg of neem kernels by the use of open-column reversed-phase liquid chromatography on Phase-bonded C-18 Hi-Flosil and high-performance liquid chromatography on μ Bondapak C-18 for monitoring (217 nm) the purification.

INTRODUCTION

Azadirachtin ($C_{35}H_{44}O_{16}$), a tetranortriterpenoid (2,3) (Fig. 1) present in neem kernels, Azadirachta indica A. Juss. (Melia azadirachta L., M. indica Brandis., Margosa tree or Indian lilac), is a potent insect antifeedant (4,5,6) and growth regulator (7). Protection from pest damage by mites, insects, and nematodes (5) has been demonstrated for azadirachtin and the crude extracts of neem seeds. Azadirachtin has not become a commonly used antifeedant due to the difficulty in isolating and purifying the

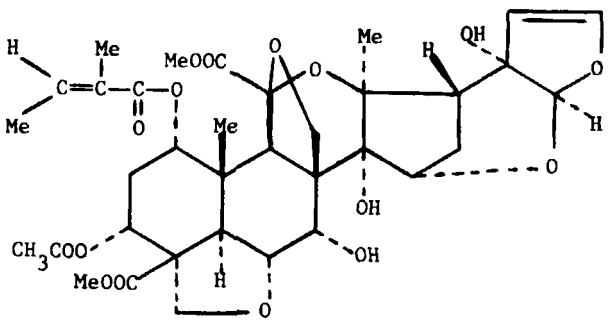

FIGURE 1 Structure of azadirachtin ($C_{35}H_{44}O_{16}$) as proposed by Zanno et al (2).

small amounts present in neem kernels. Our objectives in the present study were: 1) to obtain sufficient azadirachtin for field tests and 2) to explore procedures for preparative isolation of azadirachtin.

Butterworth and Morgan (4) isolated 1.51 g of azadirachtin by grinding 2 kg of A. indica seeds in ethanol, partitioning the extract between methanol and light petroleum, chromatographing the methanol-soluble portion on Floridin earth, and subjecting the active fractions to preparative thin-layer chromatography (TLC). Zanno et al. (3) isolated 800 mg of azadirachtin from 300 g of neem seeds by an ethanolic extraction, silica gel chromatography, and preparative TLC.

We now describe a preparative procedure for isolating azadirachtin whereby we obtained 8.7 g of the material (>90% purity) from 48.2 kg of neem seed kernels by using open-column reversed-phase chromatography with high-performance liquid chromatographic (HPLC) monitoring at 217 nm as the key step.

MATERIALS AND METHODS

Apparatus

A Waters Associates Model ALC-100 Liquid Chromatograph equipped with a Model 660 solvent programmer, two Model 6000A pumps, a U6K closed loop injector, a Buchler Fractomette 200 fraction collector, and a Schoeffel Instrument Corp. SF 770 multi-wavelength UV detector at 217 nm was used for all HPLC. The column for HPLC was μ Bondapak C-18 (30 X 0.39 cm I.D., Waters Associates).

Solvents and chromatographic materials

Hexane, acetone, and methanol were obtained from Burdick & Jackson Laboratories, Inc. (Muskegan, Michigan). Water was distilled and filtered. Diethyl ether (A.C.S. grade) was obtained from U.S. Industrial Chemicals Co. (New York, New York). Florisil 60-100 mesh was obtained from Fisher Scientific Co. (Fair Lawn, N.J.). Phase-bonded C-18 Hi-Flosil 80-100 mesh was obtained from Applied Science Laboratories, Inc. (State College, Pa.). Silica gel TLC plates (MQ65, 200 μm, 1 X 3 in) were obtained from Pierce-Quantin (Rockford, Ill.).

Plant material

Neem kernels (48.2 kg) obtained from the 1977 harvest near New Delhi, India, were ground (Fig. 2) in 1-kg batches with 2 L of hexane in a Waring Blendor (Model CB-4) for 4 min at medium speed. Each batch of homogenate was filtered through a Büchner funnel, and the residue was washed with 500 ml of hexane. The marc in 1.1-kg batches was then extracted continously for 24 h with ace-

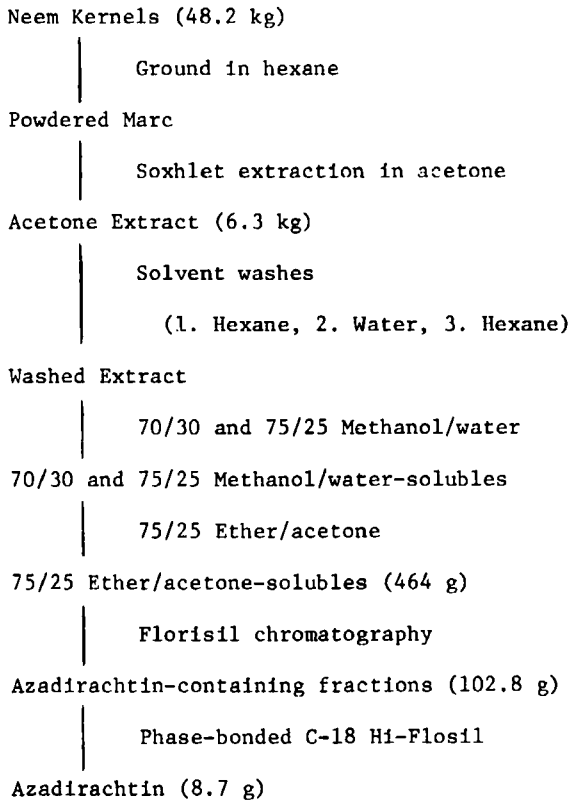

FIGURE 2 Flow diagram of the isolation of azadirachtin from neem kernels.

tone in a Soxhlet extractor, and the combined acetone extracts were concentrated to 6.3 kg.

Solvent washes

Each 200-g batch of acetone extract was washed with 1 L of hexane by stirring for about 5 min. The hexane-insoluble residue was dissolved in acetone and filtered. The combined filtrate was concentrated to yield 1.3 kg of material.

Each 200-g batch of hexane-insoluble material was washed with 1 L of distilled water. The water was decanted from a gummy insoluble material. The aqueous supernatant was filtered to give a brownish powder that was combined with the gummy insoluble material. The total combined water-insoluble material weighed 850 g.

Each 100-g batch of water-insoluble material was washed with 500 ml of hexane as before. The total combined hexane-insoluble material weighed 691 g.

Each 100-g batch of hexane-insoluble material was washed with 500 ml of 70/30 methanol/water and then with 500 ml of 75/25 methanol/water. The total combined methanol/water-soluble material from the 70/30 and 75/25 washes weighed 532 g.

Each 100-g batch of methanol/water-soluble material was washed with 500 ml of 75/25 diethyl ether/acetone. The total combined diethyl ether/acetone-soluble material weighed 464 g.

Normal phase chromatography

The acetone-soluble material (464 g) was chromatographed in 2 batches on 2.5 kg of Florisil (95 X 9.9 cm O.D.), slurry-packed in diethyl ether. An elution scheme ranging from ether to 50:50 ether/acetone was employed. Fractions containing azadirachtin were combined to give 102.8 g. Most of the azadirachtin eluted between 90/10 and 80/20 ether/acetone.

Reversed-phase chromatography

Each 25-g batch of the azadirachtin-containing fraction was chromatographed on 300 g of Phase-bonded C-18 Hi-Flosil (56 X 4.8

cm O.D.), slurry-packed in 50/50 methanol/water. The material was placed on the column in 40 ml of methanol and eluted with 50/50 methanol/water. The column was then flushed with methanol and re-equilibrated with 50/50 methanol/water for the next run. Fractions from the column that were rich in azadirachtin were then refractionated through the same column. This recycling was repeated 20 times to yield 8.7 g of >90% pure azadirachtin.

Fraction monitoring

Fractions were anlyzed for azadirachtin by HPLC as follows: Samples were dissolved in methanol and injected into a μ Bondapak C-18 column (30 X 0.39 cm I.D.) with a flow rate of 1 ml/min of 50/50 methanol/water and detection at 217 nm. Azadirachtin had an R_v of 12-13 ml. Amounts of azadirachtin present were estimated by comparing peak height with an authentic sample of azadirachtin (8).

Fractions were also analyzed for azadirachtin by TLC (9). Spots of 100 or 200 μg were developed with 50/10 diethyl ether/acetone and visualized by dipping the plates into aqueous 8% phosphoric acid-2% cupric acetate and charring on a hot plate. The R_f of authentic azadirachtin (8) was 0.65.

RESULTS AND DISCUSSION

A large quantity of azadirachtin (8.7 g) was obtained for field testing by the procedure described. The isolation was dependent upon the repetitive use of a Phase-bonded C-18 Hi-Flosil open column for reversed-phase liquid chromatography. Recycling the fractions produced a good separation between azadirachtin and contaminating materials of similar polarity;

also the column could always be reused after flushing with methanol. Azadirachtin eluted from this column between 1300 and 1500 ml of eluent with the conditions described.

The isolation procedure was also dependent upon the use of μ Bondapak C-18 for monitoring azadirachtin at 217 nm. All isolation steps and refractionations were monitored to achieve the best possible yield.

Isolation of azadirachtin by other methods described in the literature (3,4) has always involved adsorption chromatography and tedious preparative TLC. Our method, using reversed-phase chromatography, is a convenient way to isolate azadirachtin from neem kernels. Variations in yield among the methods of isolation are probably due to geographical location of the plant, time of harvest, and length of storage.

ACKNOWLEDGEMENT

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REFERENCES

1. Trade names are used in this publication solely for the purpose of providing specific information. Mention of a commercial or a proprietary product does not constitute a recommendation, guarantee, or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over the products mentioned.

2. Butterworth, J. H., Morgan, E. D. and Percy, G. R. The structure of azadirachtin; the functional groups. J. Chem. Soc. Perkin Trans., 1, 2445, 1972.

3. Zanno, P. R., Miura, I., Nakanishi, K. and Elder, D. L. Structure of the insect phagorepellent azadirachtin. Application of PRFT/CWD carbon-13 nuclear magnetic resonance. J. Am. Chem. Soc., 97, 1975, 1975.

4. Butterworth, J. H. and Morgan, E. D. Investigation of the locust feeding inhibition of the seeds of the neem tree, Azadirachta indica. J. Insect Physiol., 17, 969, 1971.

5. Warthen, J. D., Jr. Azadirachta indica: a source of insect feeding inhibitors and growth regulators. USDA, Sci, & Educ. Adm., Agric. Reviews and Manuals, Northeastern Ser. To be published.

6. Gill, J. S. and Lewis, C. T. Systemic action of an insect feeding deterrent. Nature (London) 232, 402, 1971.

7. Ruscoe, C. N. E. Growth disruption effects of an insect antifeedant. Nature New Biol. 236, 159, 1972.

8. Warthen, J. D., Jr., Redfern, R. E., Uebel, E. C. and Mills, G. D., Jr. An antifeedant for fall armyworm larvae from neem seeds. USDA, Sci. and Educ. Adm., Agric. Res. Results, Northeastern Ser. 1, 9 p., 1978, Beltsville, MD.

9. Warthen, J. D., Jr., Uebel, E. C., Dutky, S. R., Lusby, W. R. and Finegold, H. Adult house fly feeding deterrent from neem seeds. USDA Sci. and Educa. Adm., Agric. Res. Results, Northeastern Ser. 2, 11 p., 1978, Beltsville, MD.