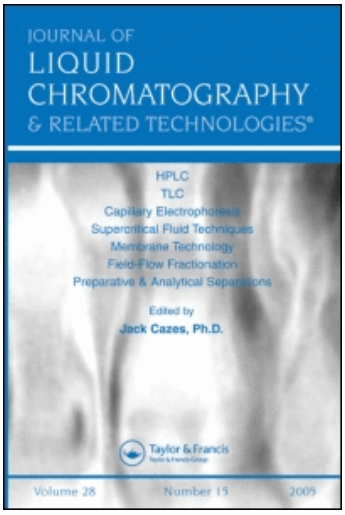

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Purification of Azadirachtin-B(3-Tigloylazadirachtol) by Preparative High Performance Liquid Chromatography, Using the Recycling Mode

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PURIFICATION OF AZADIRACHTIN-B (3-TIGLOYLAZADIRACHTOL) BY PREPARATIVE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, USING THE RECYCLING MODE

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ABSTRACT

Isolation of Azadirachtin-B in pure form suitable for crystallisation from neem seed kernel extract by a preparative high performance liquid chromatography followed by a subsequent preparative HPLC using the recycling mode is described.

INTRODUCTION

Of the numerous tetranortriterpenoids present in neem kernel extract or in neem oil, Azadirachtin-B (3-tigloylazadirachtol) is an important constituent with high biological activity and present in almost a third of the quantity of Azadirachtin-A, the principal anti-feedant and ecdysis inhibition principle.

The Azadirachtin-B of Rembold et al[1] and 3-tigloylazadirachtol of Klenk et al[2] are undoubtedly identical. However, the assignment of the tigloyl group to the 3-position is based entirely on NOE studies. Although Klenk et al[2] obtained this compound in a crystalline condition, no X-ray studies were carried out. Rembold could not crystallise Azadirachtin-B.[1] Herein, we report the purification of Azadirachtin-B suitable for crystallisation by direct preparative high performance liquid chromatography[3] of neem seed kernel extract.

MATERIALS AND METHODS

Preparative High Performance Liquid Chromatography was carried out using a Shimadzu LC8A HPLC system linked to a CR4A data processor and the peaks detected at 215 nm. Two Shimpack reverse phase (C_{18}) preparative columns (25 cm x 50 mm i.d.) and (25 cmx 20 mm i.d.) were used for preparative runs and Phenomenex reverse phase column (C_{18}) (25 cm x 4.6 mm) was used for analysis.

Neem kernel extract enriched to 25% Azadirachtin content as described earlier[3] was employed.

RESULTS AND DISCUSSION

The isolation of Azadirachtin-B by preparative high performance liquid chromatography from neem kernel extract has been reported from this laboratory,[3] but attempts to crystallise this material failed. It was surmised that material of higher purity would be needed for successful crystallisation. Hence it was decided to apply the recycling mode in preparative HPLC, which effectively increases the column length several fold, depending on the number of cycles. Also undesired accompanying impurities could be rejected using peak shaving techniques. Although recycling is a well-established procedure very little use has been made for purification of natural products.

Azadirachtin-B (500 mg) obtained as earlier[3] was subjected to one more preparative HPLC run under recycling mode using Shimadzu RP C_{18} column (25 cm x 20 mm i.d.) with 50:35:15 H_2O:MeOH: CH_3CN as eluent at 15 mL/min. flow rate (Figure 1). Peak eluting out at 16.6 min. (fraction from 11 min. to 26 min.) was recycled four times. The recycled portions are indicated by broken lines (Figure 1).

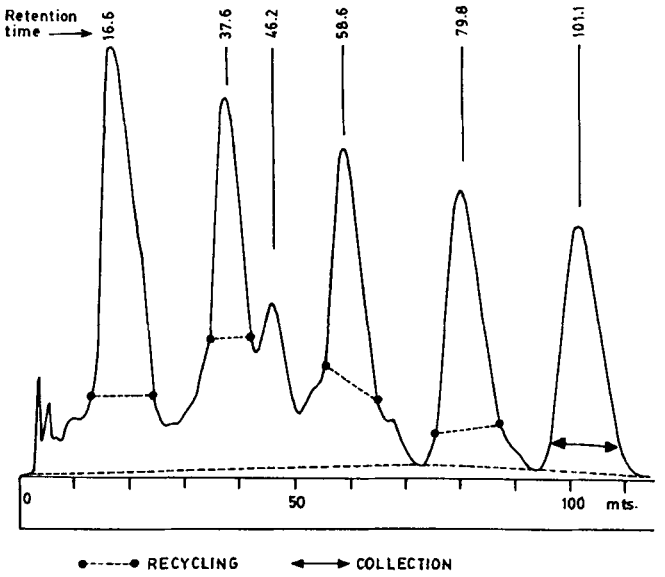

Figure 1. Preparative high performance liquid chromatogram of azadirachtin-B using recycling mode.

At the end of the fourth cycle, the peak eluting at 101.074 was symmetric and, hence, the fraction eluting out between 97 min. and 110 min. was collected and concentrated *in vacuo* to yield pure azadirachtin-B (172 mgs), which was subjected to crystallisation in various solvents (ethylacetate:hexane, methanol:water and ethanol:water), successively.

The characteristics of the crystals of azadirachtin B (EtOH-H_2O) are the following: m.pt 208°C; $[\alpha]_D$ = -76.9 (C=0.1, CH_2Cl_2); UV λ_{max} 208 nm (ε = 1.6688 x 10^4). Analysis C 59.80% H 6.38% $C_{33}H_{42}O_{14}$ requires C 59.79% H 6.39%.

The NMR data(proton and C-13) agreed completely with the data reported by Klenk et al.[1] On examination by X-ray diffraction it was found that the crystal belonged to the orthorhombic system, space group $P2_1\ 2_1\ 2_1$ with cell parameters , a = 7.646 (3) A, b = 18.599 (2) A and C = 44.671 (7) A. Determination of the structure of Azadirachtin B by X-ray diffraction is in progress and will be reported separately.

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