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Journal of Chromatography A, 1151 (2007) 203–210
JOURNAL OF CHROMATOGRAPHY A
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Purification of the seven tetranortriterpenoids in neem (*Azadirachta indica*) seed by counter-current chromatography sequentially followed by isocratic preparative reversed-phase high-performance liquid chromatography

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Available online 30 March 2007

Abstract

Counter-current chromatography (CCC) sequentially followed by isocratic preparative reversed-phase high-performance liquid chromatography was used to isolate the seven bio-actives (azadirachtin A, azadirachtin B, azadirachtin H, desacetylnimbin, desacetylsalannin, nimbin and salannin) from the seed concentrate (NSC) of the neem tree *(Azadirachta indica* A. Juss). Reproducible, narrow polarity range, high purity fractions were obtained from repeated injections of the NSC (700 mg loadings/injection), on to a relatively small volume CCC coil (116 mL). The CCC biphasic solvent system chosen was hexane:butanol:methanol:water (1:0.9:1:0.9, v/v). A mass balance of injected material showed that 95+% were recovered.

Keywords: Neem; Tetranortriterpenoids; Limonoids; Counter-current chromatography; Preparative high-performance liquid chromatography

1. Introduction

Neem (*Azadirachta indica* A. Juss) tree belongs to the Meliaceae family and it has attracted worldwide attention due to its activity against 400 insect pests [1,2]. More than 300 compounds have been characterized from neem seeds, one-third of which are tetranortriterpenoids (limonoids) [1]. Purification of neem seed limonoids has been reported with column and preparative chromatography [3–6], HPLC [7–11] and medium-pressure liquid chromatography (MPLC) [12].

Counter-current chromatography (CCC) is a liquid–liquid partition method that uses no solid support matrix [13] so irreversible column contamination does not occur, and very high percentage recoveries of compounds in unaltered form are common. CCC has been extensively applied for the separation of natural products [13–16], but to date; only three studies are reported on the utilization of CCC to separate azadirachtin A from the neem seeds [17–19]. The authors were unable to replicate some of these results [18] on the CCC preparative instrumentation utilized, which was a different form of CCC to that previously used; this is not an unusual phenomena in CCC and droplet centrifugal partition chromatography.

In this study, we report a new method to separate the seven tetranortriterpenoids (Fig. 1) in the neem seed concentrate (NSC) using CCC sequentially followed by preparative reversed-phase high-performance liquid chromatography (CCC + prep-HPLC). In this study, the following compounds were separated: azadirachtin A (dimethyl (2a*R*,3*S*,4*S*,4a*R*,5*S*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)-10-(acetyloxy)-3,5-dihydroxy-4-[(1a*R*,2*S*,3a*S*,6a*S*,7*S*,7a*S*)-6a-hydroxy-7a-methyl-3a,6a,7,7a-tetrahydro-2,7-methanofuro [2,3-*b*]oxireno[*e*]oxepin-1a(2*H*)-yl]-4-methyl-8-{[(2*E*)-2-methylbut-2-enoyl]oxy}octahydro-1*H*-naphtho[1,8a-*c*:4,5-*b*′*c*′]difuran-5,10a(8*H*)-dicarboxylate); azadirachtin B (dimethyl (2a*R*,3*S*,4*S*,4a*R*,5*S*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)-3,8-dihydroxy-4-[(1a*R*,2*S*,3a*S*,6a*S*,7*S*,7a*S*)-6a-hydroxy-7a-methyl-3a,6a,7,7a-tetrahydro-2,7-methanofuro[2,3-*b*]oxireno[*e*]oxepin-1a(2*H*)-yl]-4-methyl-10-{[(2*E*)-2-methylbut-2-enoyl]oxy}octahydro-1*H*-naphtho[1,8a-*c*:4,5-*b*′*c*′]difuran-5,10a(8*H*)-dicarboxylate); azadirachtin H (methyl (2a*R*,3*S*,4*S*,4a*R*,5*R*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)-10-(acetyloxy)-3,5-dihydroxy-4-[(1a*R*,2*S*,3a*S*,6a*S*,7*S*,7a*S*)-6a-hydroxy-7a-methyl-3a,6a,7,7a-tetrahydro-2,7-methanofuro[2,3-*b*]oxireno[*e*]oxepin-1a(2*H*)-yl]-4-methyl-8-{[(2*E*)-2-methylbut-2-enoyl]oxy}octahydro-1*H*-naphtho[1,8a-*c*:4,5-*b*

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doi:10.1016/j.chroma.2007.03.086

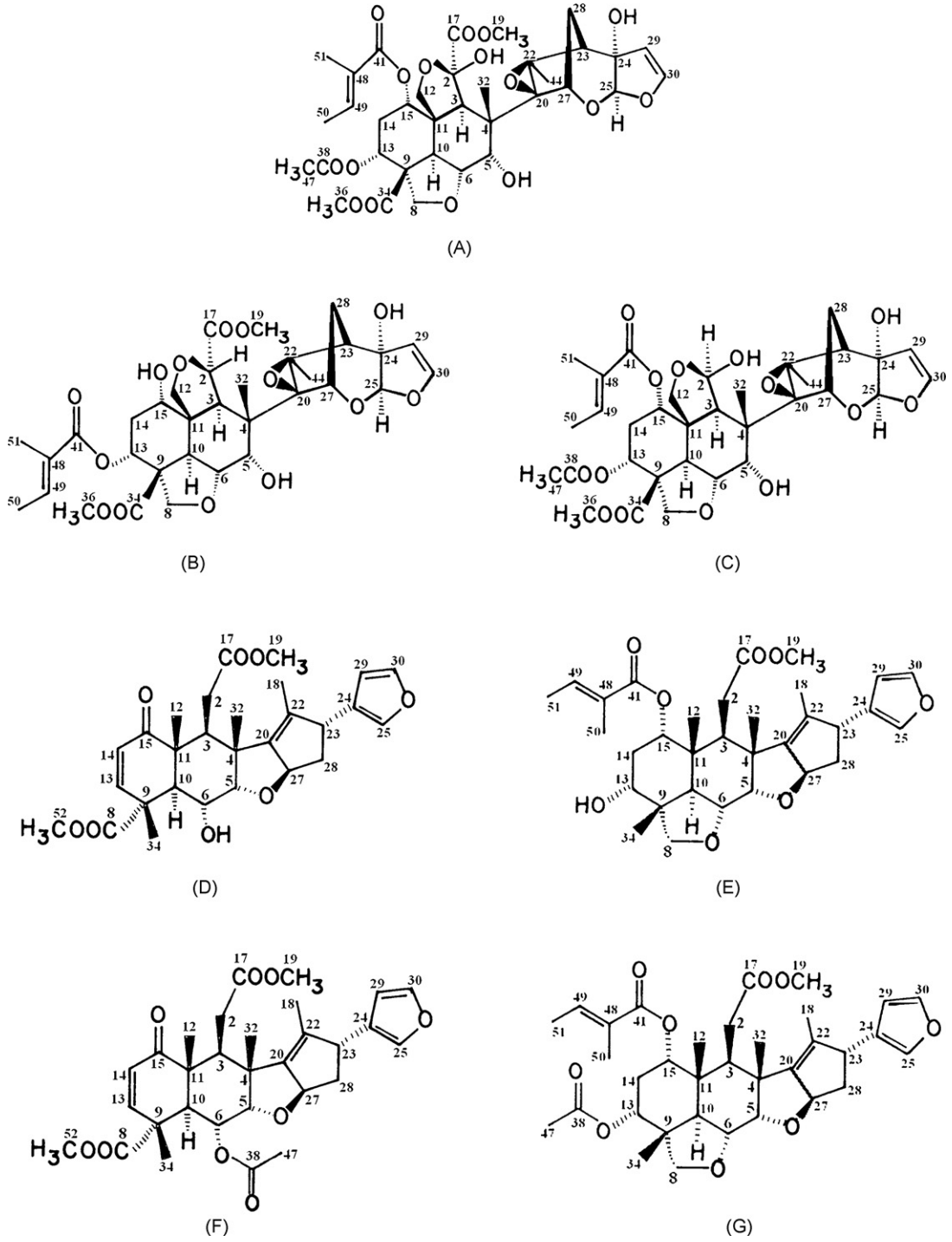

Fig. 1. Structures of tetranortriterpenoids isolated from neem seeds—azadirachtin A (A), azadirachtin B (B), azadirachtin H (C), desacetylnimbin (D), desacetylsalannin (E), nimbin (F) and salannin (G).

′*c*′]difuran-10a(8*H*)-carboxylate); desacetylnimbin (methyl (2*R*,3a*R*,4a*S*,5*R*,5a*R*,6*R*,9a*R*,10*S*,10a*R*)-2-furan-3-yl-5-hydroxy-10-(2-methoxy-2-oxoethyl)-1,6,9a,10a-tetramethyl-9-oxo-3,3a,4a,5,5a,6,9,9a,10,10a-decahydro-2*H*-cyclopenta[*b*]naphtho[2,3-*d*]furan-6-carboxylate); desacetylsalannin ((2a*R*,3*R*,5*S*,5a*R*,6*R*,6a*R*,8*R*,9a*R*,10a*S*,10b*R*,10c*R*)-8-furan-3-yl-3-hydroxy-6-(2-methoxy-2-oxoethyl)-2a,5a,6a,7-tetramethyl-2a,4,5,5a,6,6a,8,9,9a,10a,10b,10c-dodecahydro-2*H*,3*H*-cyclopenta[*d*]naphtho[2,3-*b*:1,8-*b*′*c*′]difuran-5-yl(2*E*)-2-methylbut-2-enoate); nimbin (methyl (2*R*,3a*R*,4a*S*,5*R*,5a*R*,6*R*,9a*R*,10*S*,10a*R*)-5-(acetyloxy)-2-furan-3-yl-10-(2-methoxy-2-oxoethyl)-1, 6,9a, 10a-tetramethyl-9-oxo-3,3a,4a,5,5a,6,9,9a,10,10a-decahydro-2*H*-cyclopenta[*b*]naphtho[2,3-*d*]furan-6-carboxylate) and salannin ((2a*R*,3*R*,5*S*,5a*R*,6*R*,6a*R*,8*R*,9a*R*,10a*S*,10b*R*,10c*R*)-3-(acetyloxy)-8-furan-3-yl-6-(2-methoxy-2-oxoethyl)-2a,5a,6a,7-tetramethyl-2a,4,5,5a,6,6a,8,9,9a,10a,10b,10c-dodecahydro-