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OPTIMISED ISOLATION PROCEDURE FOR BIOLOGICALLY ACTIVE COMPOUNDS NIMBOLIDE AND 28-DEOXONIMBOLIDE FROM *AZADIRACHTA INDICA* LEAVES

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Key Word Index—*Azadirachta indica*; meliaceae; leaves; α-linolenic acid; nimbolide; 28-deoxonimbolide; limonoids.

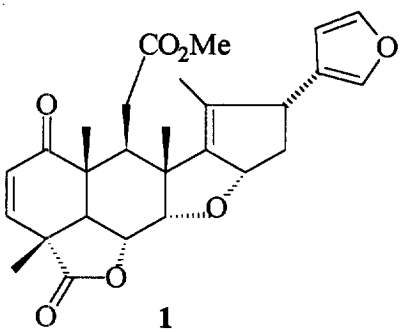
Abstract—α-linolenic acid has been isolated from fresh *A. indica* leaves and an optimised procedure for the isolation of the limonoids nimbolide and 28-deoxonimbolide is described. © 1997 Elsevier Science Ltd

INTRODUCTION

Azadirachta indica A. Juss, commonly called the 'neem tree' in India has been investigated in detail during the last three decades and a large number of biologically active compounds have been isolated from it [1]. The best known among them is azadirachtin, the structure of which was confirmed by Ley *et al.* [2]. The Indian traditional medicine 'Ayurveda' makes extensive use of all parts of *A. indica* [3]. Dry *A. indica* leaves have been traditionally used by Indian farmers to protect grains and legumes from storage pests [4]. One of the major compounds found in the leaves is nimbolide **1**, which was first isolated from fresh leaves by Ekong *et al.* [5]. Later, isolation and structural elucidation using ^{1}H and ^{13}C NMR data of **1** and 28-deoxonimbolide **2** was carried out by Kraus and associates [6]. A recent report by Rochanakij *et al.* [7] showed that **1** possessed an antimalarial effect.

Extensive studies conducted at RRL, Trivandrum, a few years ago, established the efficacy of neem leaf extract as an antifeedant for the control of two pests, viz. *Longitarsus nigripennis* (Coleoptera, a pest of black pepper commonly called 'Pollu beetle') and *Heliopeltis antonii* (Coleoptera, a pest of the cashew tree commonly called 'tea mosquito bug'). This finding was of significance as commercially available azadirachtin-containing antifeedants did not show much effect on *L. nigripennis*. Nimbolide and 28-deoxonimbolide were found to be the major components in the antifeedant fraction [8].

In view of its therapeutic and antifeedant effects, development of an extraction protocol designed for obtaining large quantities of **1** and **2** was sought. While Ekong [5] reported the isolation of **1** from fresh neem leaves using hexane as solvent, the com-



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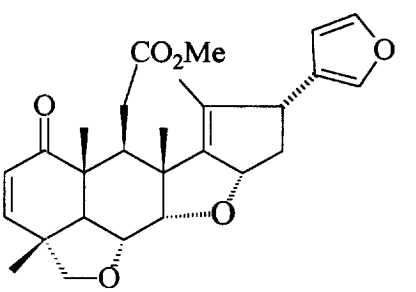

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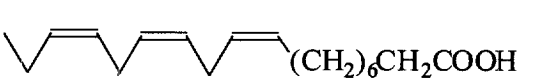

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munication did not contain further details. Bokel *et al.* [6] isolated **1** and **2** from dry leaves using diethyl ether extraction followed by repeated chromatographic separations including reversed-phase chromatography.

After extensive studies using fresh and dry leaves with various solvents, we have been able to identify the best conditions for obtaining **1** and **2**. These studies have also led to the isolation of the nutrient, α-linolenic acid **3** from fresh *A. indica* leaves. Dry leaf extract did not contain α-linolenic acid.

RESULTS AND DISCUSSION

Thin layer chromatography of dry *A. indica* leaf extract with hexane indicated the presence of a large number of compounds compared to that of fresh leaves. Extraction of *A. indica* leaves shade-dried for 48 hr (temp. ≈30°, average loss of water 18–20%) with hexane was found to be most suitable in terms of total yield, ease of separation and best yields of **1**, **2** and **3**. After chromatographic removal of very low polarity compounds and carotenes, α-linolenic acid and palmitic acid were obtained as a mixture. Separation was carried out by conversion to the methyl ester followed by chromatography on $AgNO_3$-impregnated silica gel. Further elution provided nimbolide and 28-deoxonimbolide which were identified by comparison of mp, mass and NMR spectra.

EXPERIMENTAL

General. All mps are uncorrected and were determined on a Melttemp II hot stage melting point apparatus. ^1H (90 MHz) and ^{13}C (22.4 MHz) NMR spectra were recorded using tetramethylsilane as an int. standard.

Extraction and isolation. 545 g of finely cut leaves of *A. indica* shade-dried for 48 hr was extracted with hexane in a Soxhlet apparatus for 24 hr. Hexane was removed under red. pres. to yield 6.63 g of crude extract which was subjected to CC on silica gel (270 g). Elution with hexane removed fats and carotenes. Further elution with 10% EtOAc–hexane gave 1.1 g of a mixt. of α-linolenic acid and palmitic acid. Elution with 25% EtOAc–hexane provided 0.716 g of nimbolide {crystallised from CH_2Cl_2–hexane mixt., mp = 245° (lit. [6] mp = 245–247°), MS, ^1H and ^{13}C NMR data identical with the reported values] and 0.525 g of 28-deoxonimbolide (mp = 170°, ^1H and ^{13}C NMR data identical with reported values [6]}.

The acid mixt. was treated with MeOH and *p*-toluenesulphonic acid and stirred at room temp. for 16 hr MeOH was removed under red. pres. and the product extracted with CH_2Cl_2. Removal of solvent followed by CC on $AgNO_3$-impregnated silica gel provided 0.225 g of methyl palmitate and 0.711 g of the methyl ester of α-linolenic acid. TLC, MS, ^1H NMR spectrum were identical with that of an authentic sample of methyl linolenate obtained from Aldrich.

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