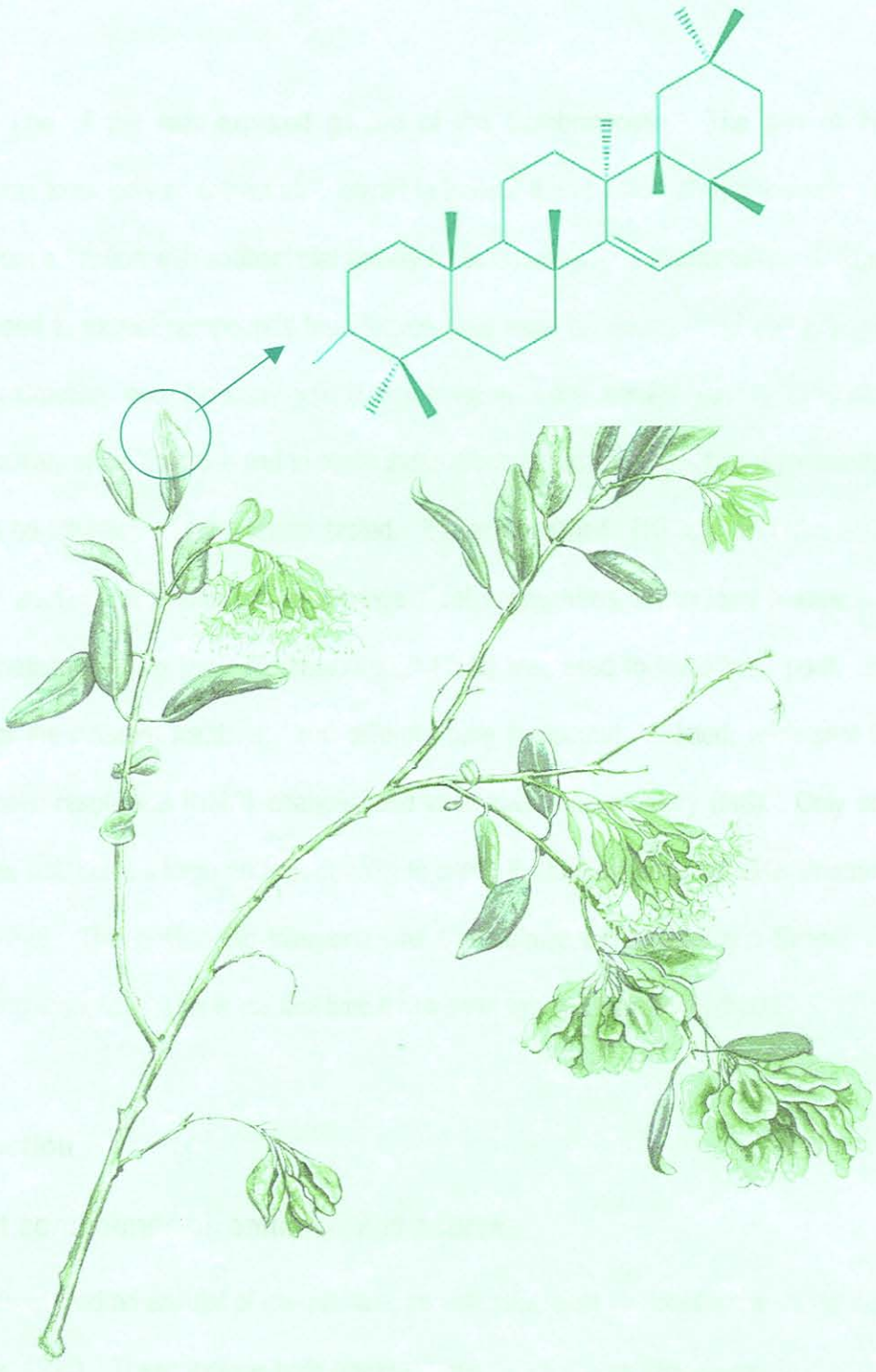
Chapter 7



Chapter 7

Column chromatography and isolation of pure compounds from *Pteleopsis myrtifolia*

Abstract

Pteleopsis is one of the less explored genera of the Combretaceae. The aim of this investigation was to simplify an extract of *P. myrtifolia* leaves, thereby facilitating the isolation of compounds from a fraction with antibacterial activity by bioassay-guided fractionation. Acetone was initially used to extract compounds from leaves (that were previously dried and ground). Liquid-liquid extraction and bioassay-guided fractionation were carried out to determine antibacterial activity of the fractions and to purify those that had activity. The chloroform fraction was found to be inhibitory to all bacteria tested. It was quantified (10.38g) and chromatographed over a silica gel column, using a range of solvent mixtures, starting with hexane and ending with methanol. Thin layer chromatography (TLC) was used to investigate purity and composition of the different fractions. The different pure compounds isolated, were sent for nuclear magnetic resonance (NMR) analysis, and later mass spectrometry (MS). Only one compound was isolated in a large enough quantity to clarify the two-dimensional NMR structure: 14-Taraxeren-3-ol. This pentacyclic triterpene was first isolated by Burrows and Simpson in 1938 from *Taraxacum* root. This is the first time it has been isolated from P. *myrtifolia*.

7.1 Introduction

7.1.1 Plant compounds as antimicrobial sources

Plants have developed an arsenal of chemicals to survive attacks by microbial invasion (Grayer and Harborne, 1994). These include both physical barriers as well as chemical ones, i.e. the

presence or accumulation of antimicrobial metabolites. These metabolites are either preformed in the plant (prohibitins) or induced after infection (phytoalexins). Since phytoalexins can also be induced by abiotic factors such as UV irradiation, they have been defined as 'antibiotics' formed in plants via a metabolic sequence induced either biotically or in response to chemical or environmental factors (Martini, 2002). When infection or damage to a plant takes place, a number of processes are activated and some of the compounds produced become activated immediately whereas phytoalexins are produced after two to three days. Sometimes it is difficult to determine whether the compounds are phytoalexins or prohibitins especially as the same compound may be a preformed antimicrobial substance in one species and a phytoalexin in another. The chemical classes in which these substances can be found vary greatly (Cowan, 1999).

In recent studies, several antibacterial compounds were isolated in the Combretaceae plant family, some for the first time in this family. One of these studies reported the isolation of the stilbene 2',3,4-trihydroxyl,3,5,4'-trimethoxybibenzyl (combretastatin B5) from *C. woodii*. This is the first report of antimicrobial activity of combretastatin B5 (Famakin, 2002).

Pteleopsis myrtifolia Engl. et Diels (Combretaceae) is distributed in KwaZulu-Natal and Limpopo (in South Africa), as well as in Botswana, Zambia, Malawi, Tanzania and other parts of Africa. In West Africa, a decoction of *P. hylodendron* 's root is popularly used for venereal diseases (Ngounou *et al.*, 1999). In folk medicine, *P suberosa* is used for its antiulcer properties (De Pasquale *et al.*, 1995). A decoction of the bark is taken orally three times a day; or sometimes the powdered bark is chewed and swallowed. The methanolic extracts had antimicrobial activity against some microorganisms that are responsible for skin infections (*Staphylococcus aureus*, *S. capitatis*, *S. epidermidis*, *S. saprophyticus*, *Bacillus subtilus*, *Pseudomonas aeruginosa*, *P. cepacia*, *Cochlospermum tinctorium* but not against *Escherichia coli*, *Proteus vulgaris* and *P. mirabilis*). The antibacterial activity ascertained, might justify the

traditional use of these plants in folk medicine for treatment of skin diseases (Bisignano *et al.*, 1996).

Antibacterial activity in leaf extracts from *P. myrtifolia* was described in Chapter 3. An acetone extract of *P. myrtifolia* leaves was separated into fractions (by group fractionation) of which the antibacterial activity was determined (Chapter 4). The chloroform fraction (which showed antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa* and *Escherichia coli*) was chosen for further investigation. The aim of this investigation was to isolate antibacterial compounds from the chloroform fraction via column chromatography, and to elucidate the structure thereof by nuclear magnetic resonance (NMR) and mass spectrometry (MS).

7.2 Materials and Methods

7.2.1 Plant material

Plant material was collected and prepared as described in 2.2.1 of Chapter 2.

7.2.2 Column chromatography

The chloroform fraction of a liquid-liquid separation of *P. myrtifolia* leaves (Chapter4) was found to have antibacterial activity against *Staphylococcus aureus* American Type Culture Collection [ATCC29213], *Enterococcus faecalis* [ATCC29212], *Pseudomonas aeruginosa* [ATCC 25922] and *Escherichia coli* [ATCC 27853].

This fraction was dried, quantified (10.38 g) and placed on a silica gel column. The column was packed according to the ratios (120-80 g/1 g plant material) described by Houghton and Raman (1998) and 8 g silica gel was used for each gram dry plant material. The silica gel was weighed, wettened with hexane, poured into a glass column and left overnight to settle. Elution

of the column was started with 100% *n*-hexane. 5% chloroform was added to the *n*-hexane, then 10%, followed by 20%. The percentage of chloroform was gradually increased to 100%. Methanol was added to the eluent (chloroform) in the same manner as chloroform was added to *n*-hexane, with gradual increments. At an eluent mixture of 40% methanol (and 60% chloroform), silica gel started eluting as well and the column was stopped. Each fraction's original volume, which varied between 100 and 400ml, was documented.

The fractions were air dried at room temperature. As they became more concentrated, two layers formed, often with slightly different colours. The two layers were separated into separate containers and tested for pure compounds on TLC plates. Thereafter, the dried fractions were each washed in hexane, methanol and chloroform and each fraction obtained from these washes were tested on TLC plates again. In total, there were 93 fractions. Since most of these were each separated into two sub fractions, approximately 186 test tubes were dried and each washed with hexane, methanol and chloroform. Crystals from test tubes were also separated according to where they dried, on the sides or bottom of the tubes, and kept in separate tubes. Each of these approximately 558 tubes' contents was tested for purity by TLC. Test tube washes that had single bands and the same R_f values were combined. These gave two pure compounds, which were dissolved in deuterated chloroform and sent for analysis with nuclear magnetic resonance (NMR). NMR data were obtained at 300 MHz on a Bruker ARX 300 NMR spectrometer using $CDCl_3$ as solvent with tetramethylsilane (TMS) as internal standard.

From chromatograms, fractions with similar separation patterns on TLC plates (more than one band) were recombined to form 13 recombined fractions (Figure 7.3). Each of these was each run on a smaller Sephadex LH-20 column with the same eluents as the silica gel column. The fractions from Sephadex columns were again washed with hexane, methanol and chloroform and tested for its composition with TLC.

From the smaller Sephadex columns, fractions or fraction combinations that gave single bands (representing possible pure compounds), 17 in total, were sent for NMR analysis.

A second crude column was run from 500mg of dried leaf material. This time, Sephadex G 100 was used as a packing material with the objective to obtain a larger amount of pure compound in the fractions. The Sephadex column was eluted with acetone and gradually methanol. The 31 fractions were recombined into 10 fractions and each developed again in smaller Sephadex columns.

Tables were drawn from ^{13}C NMR results and compared to other natural compounds described in literature. The pure compounds were sent for mass spectrometry.

7.3 Results and Discussion

7.3.1 First crude column

As the column's elution started, compounds started to be separated and moved down the column at different rates (Figure 7.1).

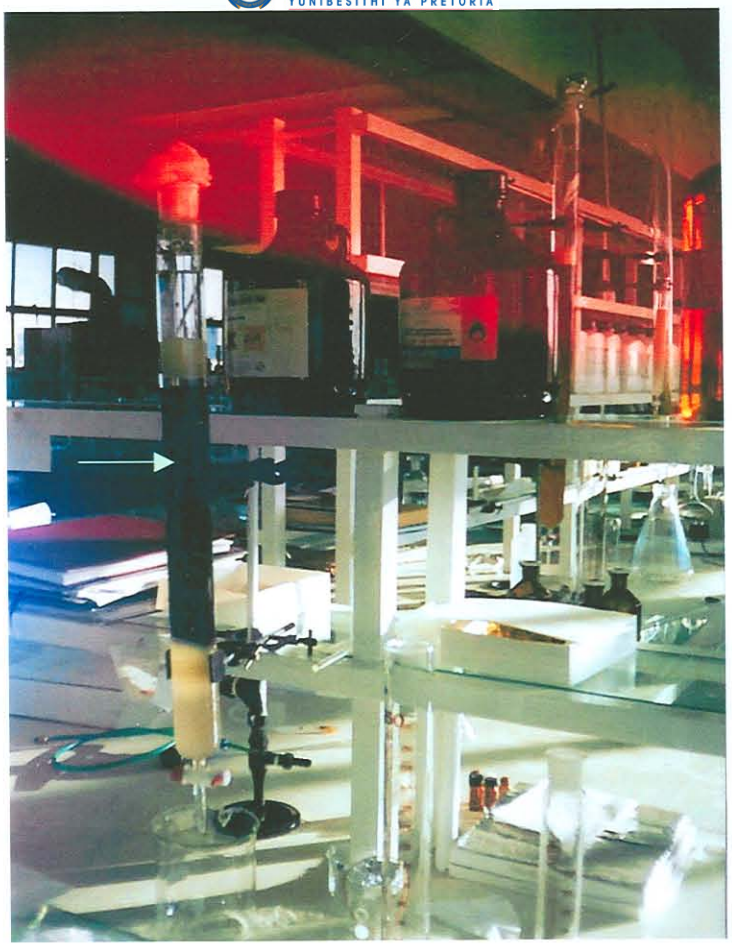


Figure 7.1. Photo of a silica gel column while eluting the chloroform fraction of *Pteleopsis myrtifolia* leaves with a mixture of hexane and chloroform (4:6).

None of the original column fractions were pure. Some of the *n*-hexane, methanol and chloroform washes (of each fraction) were pure.

When a thin layer chromatogram of every tenth fraction from the column fractions was developed, one could see that the non-polar fractions were less complex than the ones of intermediate polarity and the polar ones. The fractions of intermediate polarity and polar ones, gave several bands with TLC (Figure 7.2).

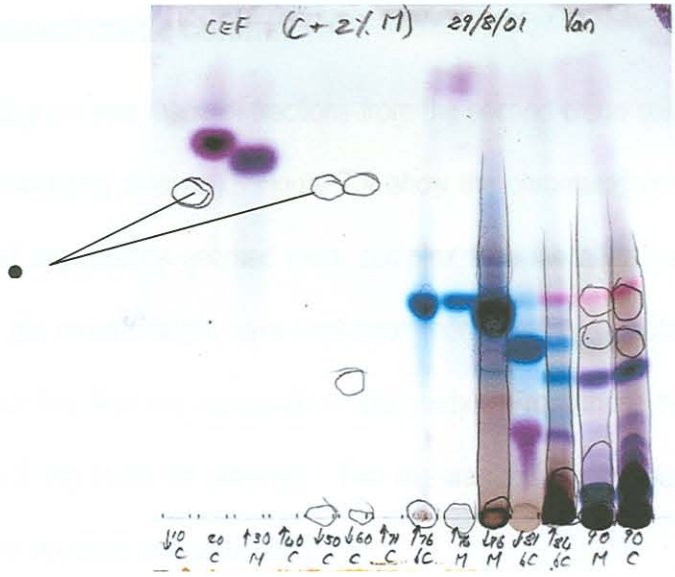


Figure 7.2. Thin layer chromatogram of very tenth fraction of column, sprayed with 0.34% vanillin in 3.5% sulphuric acid in methanol. ● = Bands that did not stain with the vanillin spray.

The 13 recombined fractions (Figure 7.3) each given an alphabet number; from left (non-polar) to right (polar), they were: E, D, F, G, H, I, J, K, L, M, N, C, B, A.

Figure 7.3. Thin layer chromatogram of the 13 combined fractions of *Pteleopsis myrtifolia* leaves, sprayed with 0.34% vanillin in 3.5% sulphuric acid in methanol.

7.3.2 Second crude column

A chromatogram was made of fractions from the second crude column, (where Sephadex was used as packaging material). Figure 7.4 show the chromatogram after sprayed with vanillin. The overall appearance seemed more complex than the silica gel column's chromatograms. The silica gel column might have kept back more hydrophilic substances than the Sephadex column (but this was not measured in this study). From these fractions, no pure compound more than 2 mg could be obtained. Two mg was too small a quantity to determine the true identity and structure of a compound.

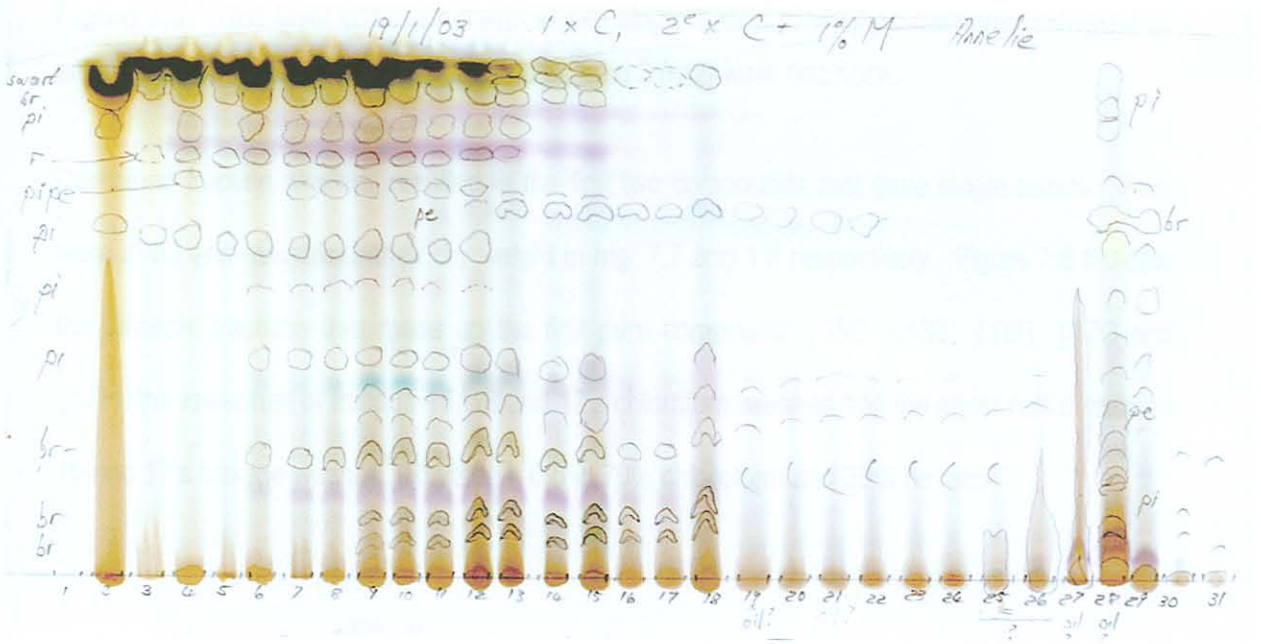


Figure 7.4. Thin layer chromatogram of fractions of second crude column (Sephadex), sprayed with 0.34% vanillin in 3.5% sulphuric acid in methanol.

7.3.3 Pure and recombined fractions

The lower half of fraction 15, 16 and 17's chloroform washes and the lower half of fraction 16 and 17's hexane washes gave single bands with a R_f value of 0.2. So also, the upper half of fraction 31 and 32's chloroform washes gave single bands as well, with an R_f of 0.88 (Figure 7.5).

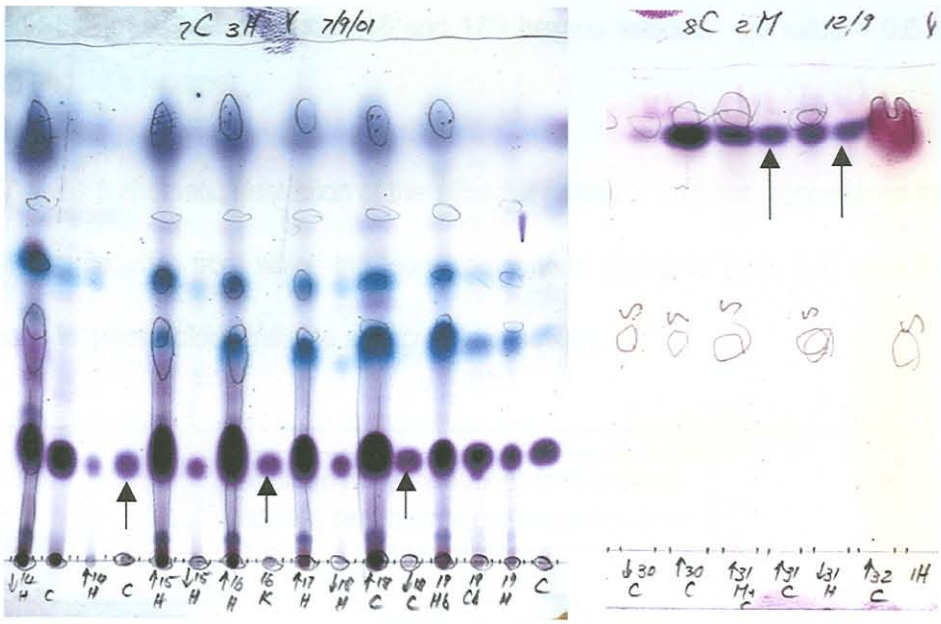


Figure 7.5. Thin layer chromatograms where single bands of washed fractions (indicated by arrows) of compound 1 (ar1) (left) and compound 2 (ar2) were first seen.

Combined fraction washes, resulted in the first two compounds that gave single bands. They were dried and quantified; their dry weight in mg: 7.7 and 1.8 respectively. Figure 7.6 indicate the different fractions that made up the first pure compound: ↓15C, ↓16C, ↓16H, ↓17C and ↓17H (the lower half of fraction 15, 16 and 17's chloroform washes and the lower half of fraction 16 and 17's hexane washes. (R_f value = 0.6 in 70% chloroform and 30% hexane)

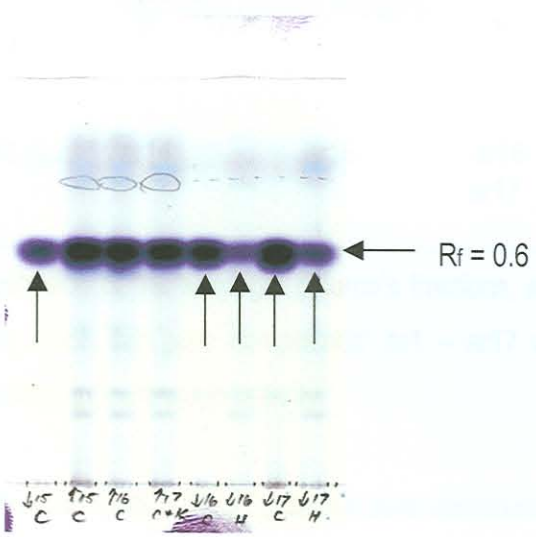


Figure 7.6. Chromatogram of bota ar**1**, developed from column fractions that were washed: ↓15C, ↓16C, ↓16H, ↓17C and ↓17H (the lower half of fraction 15, 16 and 17's chloroform

washes and the lower half of fraction 16 and 17's hexane washes. (R_f value = 0.6 in 70% chloroform and 30% hexane)

Figure 7.7 gives a shematic illustration of the silica gel column's fractions, recombined fractions and Sephadex columns from which the 'pure compounds' (listed in Table 7.1) were isolated. The increase in green colour, indicate an increase in polarity.

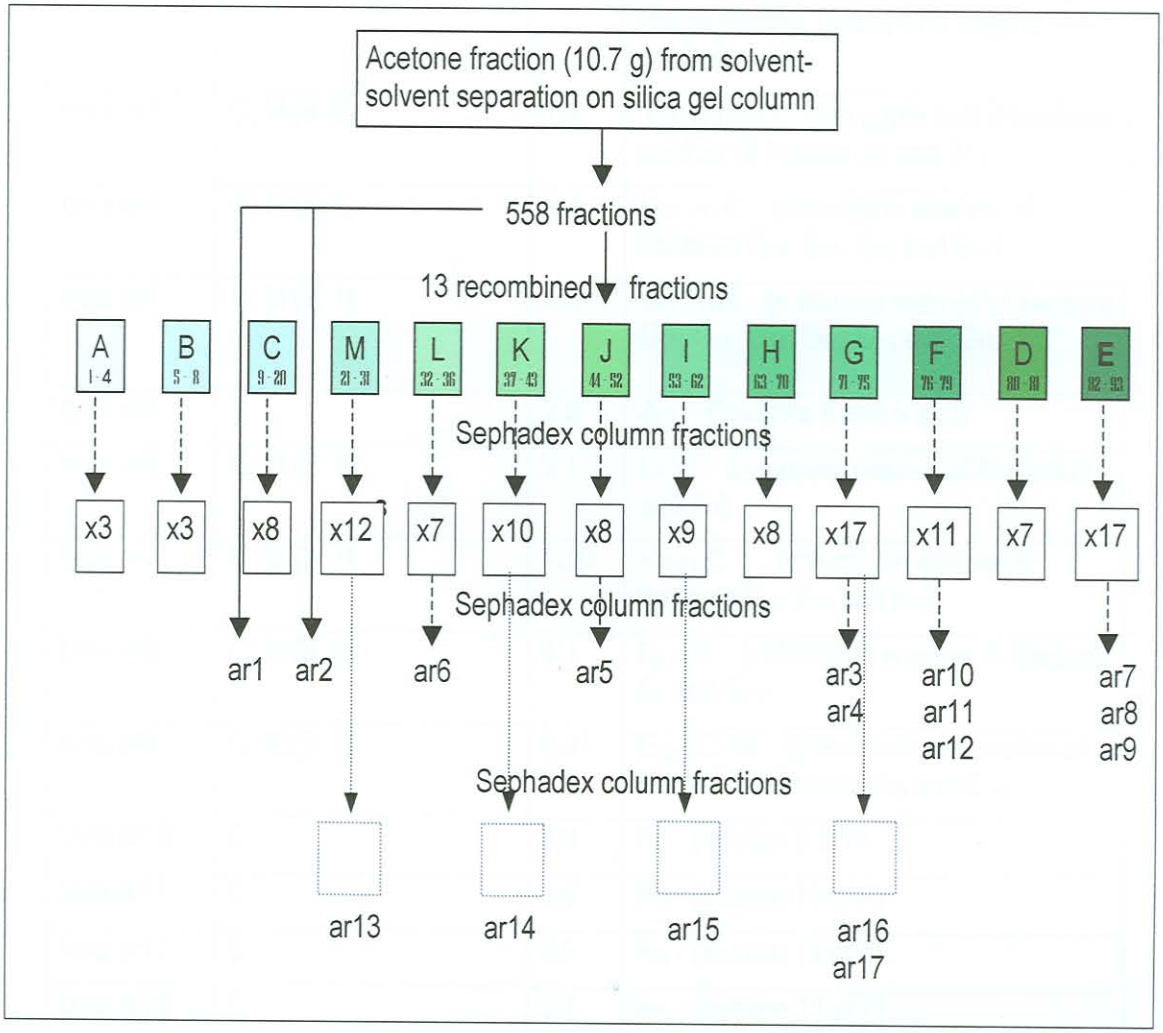


Figure 7.7. A schematic illustration of the silica gel column's fractions, recombined fractions and Sephadex columns from which the 'pure compounds' ar1 – ar17 were isolated. The increase in green colour, indicates an increase in polarity.

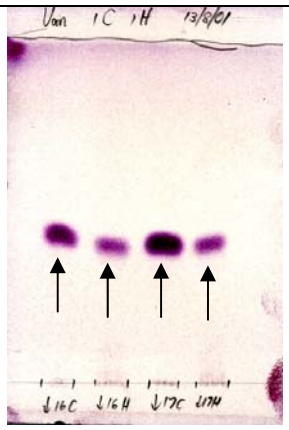
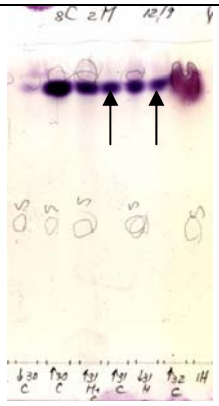
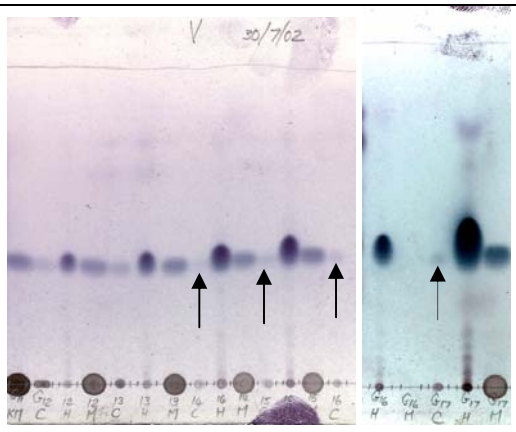
Table 7.1 lists and explains the composition the 17 possible pure compounds (from fractions of the crude column as well as from Sephadex columns run from recombined fractions) that were sent for NMR analysis, and Table 7.2 show their single bands.

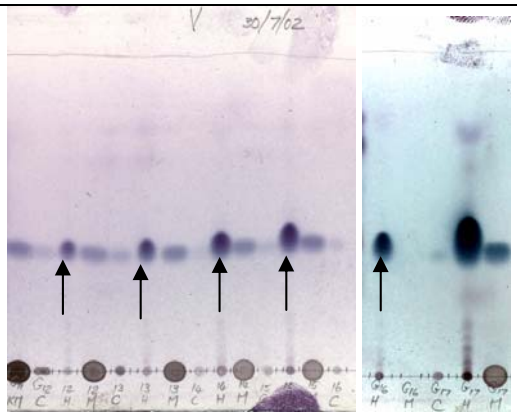
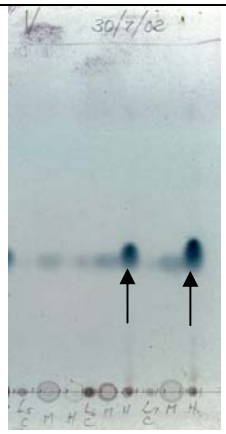
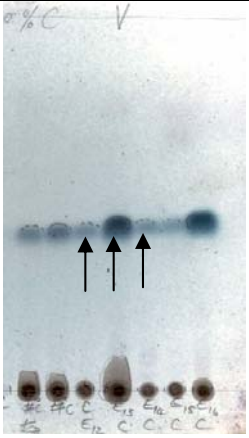
Table 7.1. Table of possible pure compounds, listed from left to right: the NMR tube number, the eluent ratio the chromatogram was developed with, milligram of material (m) and composition (fraction numbers). The deuterated solvent for all compounds was chloroform.

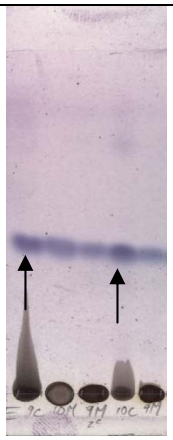
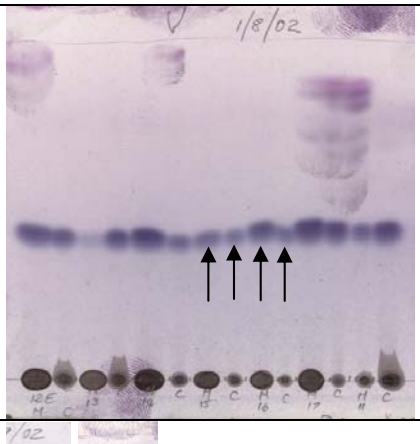
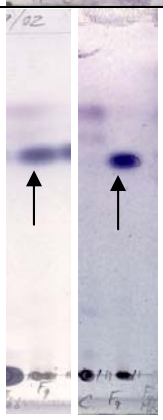
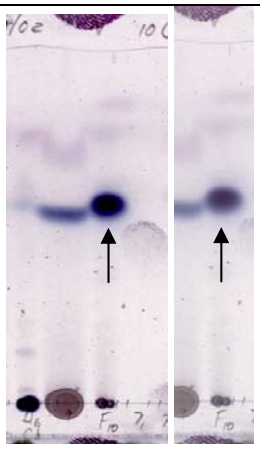
Tube number for NMR	Eluent (ratio) chromatogram was developed with:	Mg m	Fraction numbers
bota ar1*	C: M (8: 2)	7.7	↓15C↓16C↓16H↓17C↓17H (the lower half of fraction 15, 16 and 17's chloroform washes and the lower half of fraction 16 and 17's hexane washes)
bota ar2	C: M (8: 2)	1.8	↑31C↑32C (the upper half chloroform washes of fraction 30 and 31)
bota ar3	C: M (9: 1)	5.4	G_{14-17} C (chloroform washes of fractions G_{14}, G_{15}, G_{16} and G_{17})
bota ar4	C: M (9: 1)	4.3	G_{12-17}H (*n*-hexane washes of fractions G_{12}, G_{13}, G_{14}, G_{15}, G_{16} and G_{17})
bota ar5	C	2.0	$J_{8,9}$ (fractions 8 and 9 of J)
bota ar6	C: M (9: 1)	2.1	$L_{6,7}$H (*n*-hexane washes of fractions L_6 and L_7)
bota ar7	C: M (9: 1)	12.0	$E_{12\text{-}14}$ C (chloroform washes of fractions E_{12}, E_{13} and E_{14})
bota ar8	C: M (9: 1)	4.1	$E_{9,10}$ C (chloroform washes of fractions E_9 and E_{10})
bota ar9	C: M (9: 1)	9.3	$E_{15,16}$C+M (chloroform and methanol washes of fractions E_{15} and E_{16})
bota ar10	C	3.8	F_9 (fraction 9 of F)
bota ar11	C	3.6	F_{10} (fraction 10 of F)
bota ar12	C	2.6	F_{11} (fraction 11 of F)
bota ar13	C	2.7	4_1 (fraction 11 of F)
bota ar14	C	3.5	$6_{0,1,6}$ (fractions 0, 1 and 6 of 6)
bota ar15	C	3.9	7_2 (fraction 2 of 7)
bota ar16	C	3.4	$3_{6,7,8}$ (fractions 6,7 and 8 of 3)
bota ar17	C	3.3	$4_{5,6,7}$ (fractions 5, 6 and 7 of 4)

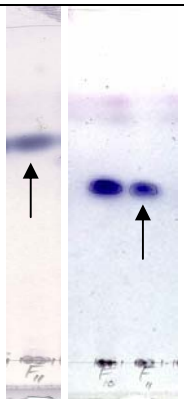
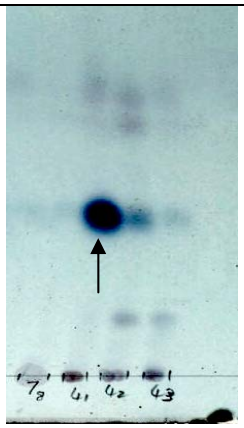
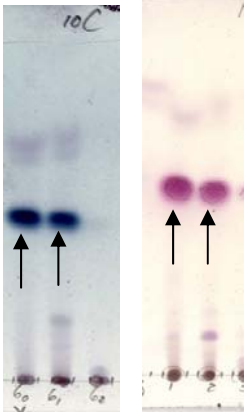
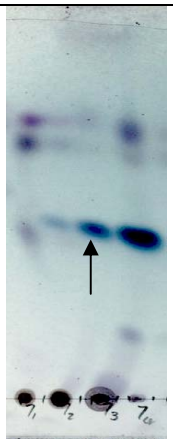
* Needle-like crystals drifted on top of the deuterated chloroform and dissolved with difficulty. (H = *n*-hexane C = chloroform, M = methanol).

Table 7.2. Table of possible pure compounds, listed from left to right: the NMR tube number and the eluent ratio the chromatogram was developed with (bottom label of each row's first column), milligram of material (m) and composition (fraction numbers). The deuterated solvent for all compounds was chloroform.

Tube no	Chromatogram	Mg m	Fractions
bota ar1 C: M (8: 2)	 C* *Needle like crystals dissolved with difficulty	7.7	↓15C↓16C ↓16H↓17C ↓17H
bota ar2 C: M (8: 2)		1.8	↑31C↑32C
bota ar3 C: M (9: 1)		5.4	G_{14-17} C

Tube no	Chromatogram	Mg m	Fractions
bota ar4 C: M (9: 1)		4.3	$G_{12-16}H$
bota ar5 C		2.0	$J_{7,8}$
bota ar6 C: M (9: 1)		2.1	$L_{6,7}H$
bota ar7 C: M (9: 1)		12.0	$E_{12-14}C$

Tube no	Chromatogram	Mg m	Fractions
bota ar8 C: M (9: 1)		4.1	$E_{9,10}$ C
bota ar9 C: M (9: 1)		9.3	$E_{15,16}$ C +M
bota ar10 C		3.8	F_9
bota ar11 C		3.6	F_{10}

Tube no	Chromatogram	Mg m	Fractions
bota ar12 C		2.6	F_{11}
bota ar13 C		2.7	4_1
bota ar14 C		3.5	$6_{0,1,2}$
bota ar15 C		3.9	7_2

Tube no	Chromatogram	Mg m	Fractions
bota ar16 C		3.4	3 $_{6,7,8}$
bota ar17 C		3.3	4 $_{5,6,7}$

The compounds were all send for NMR analysis - firstly the proton spectrum, then the ^{13}C spectrum and lastly 2-dimensional spectra. After each spectrum, a compound was re-evaluated for purity and structure type.

7.3.4 Nuclear magnetic resonance

Proton spectra of NMR analysis indicated structures of terpenoids for the first two (Figures 7.8 and 7.9) and twelfth (Figure 7.10) pure compounds. The ^{13}C NMR graph of the pure compound bota ar1 is shown in Figure 7.11. The two-dimensional NMR graphs, heteronuclear multiple quantum correlation (HMQC) and correlated spectroscopy (COSY) of bota ar1 is shown in Figures 1.12 and 7.13 respectively.

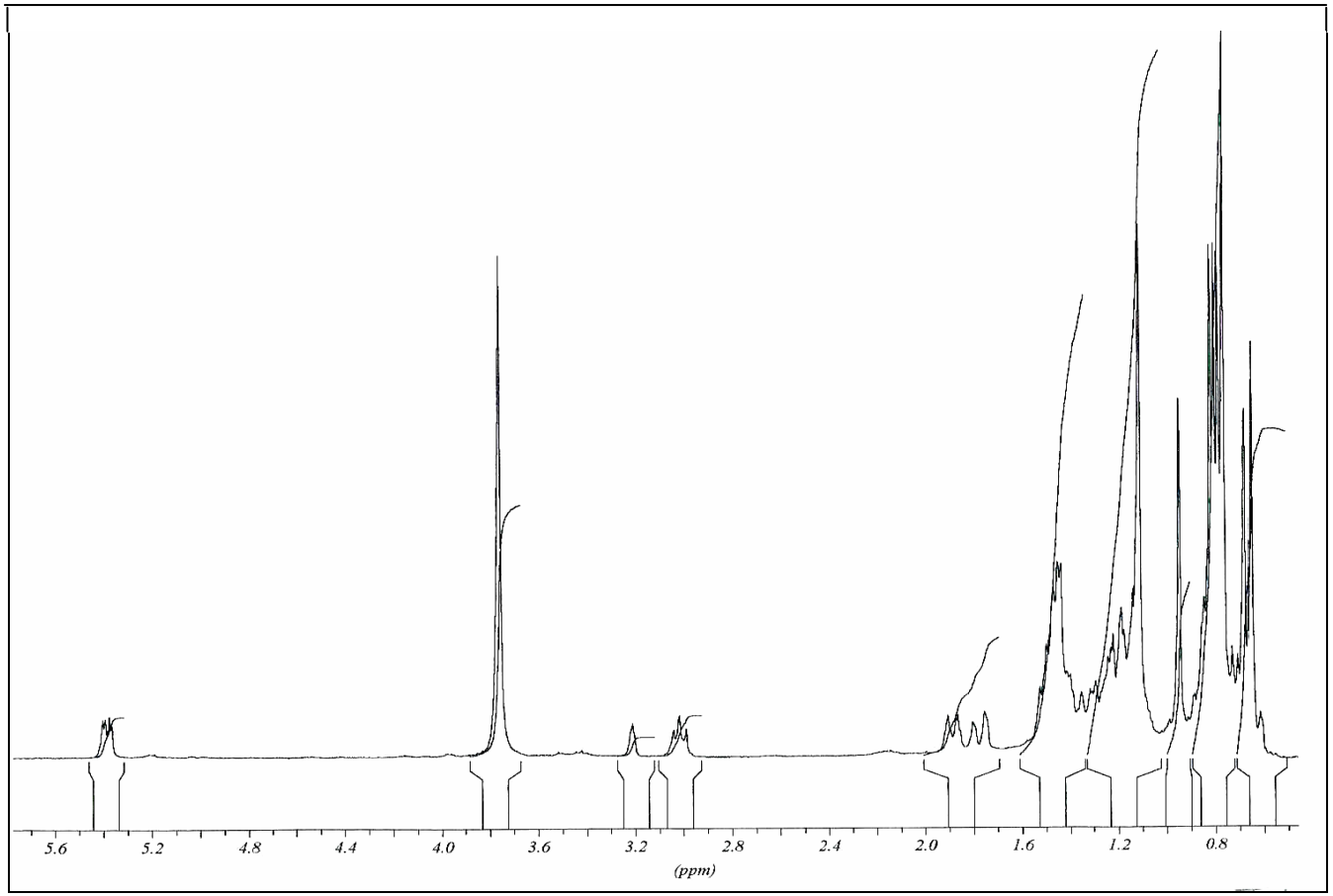


Figure 7.8. The proton spectra from NMR analysis of the first (bota ar1) pure compound.

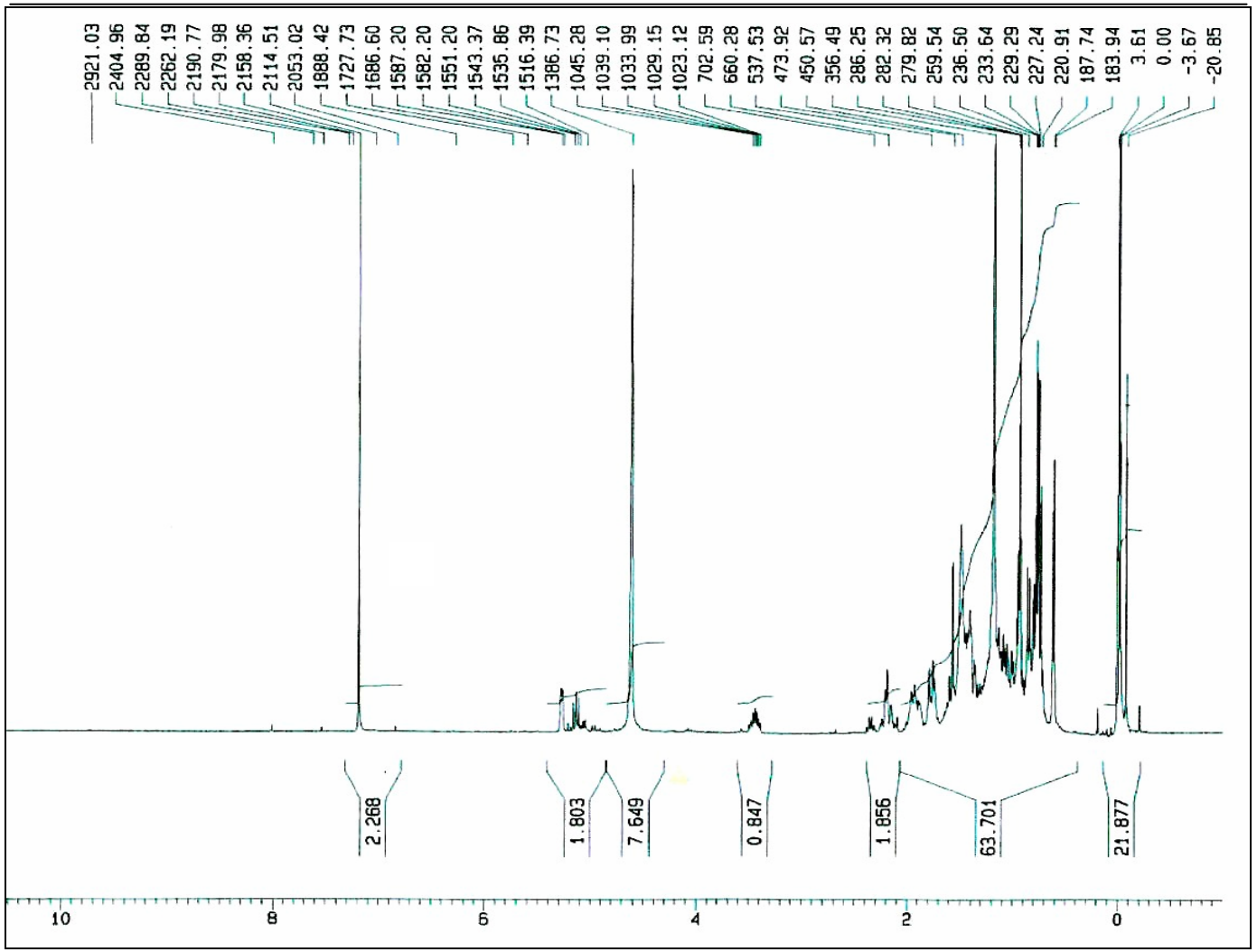


Figure 7.9. The proton spectra from NMR analysis of the second (bota ar2) pure compound.

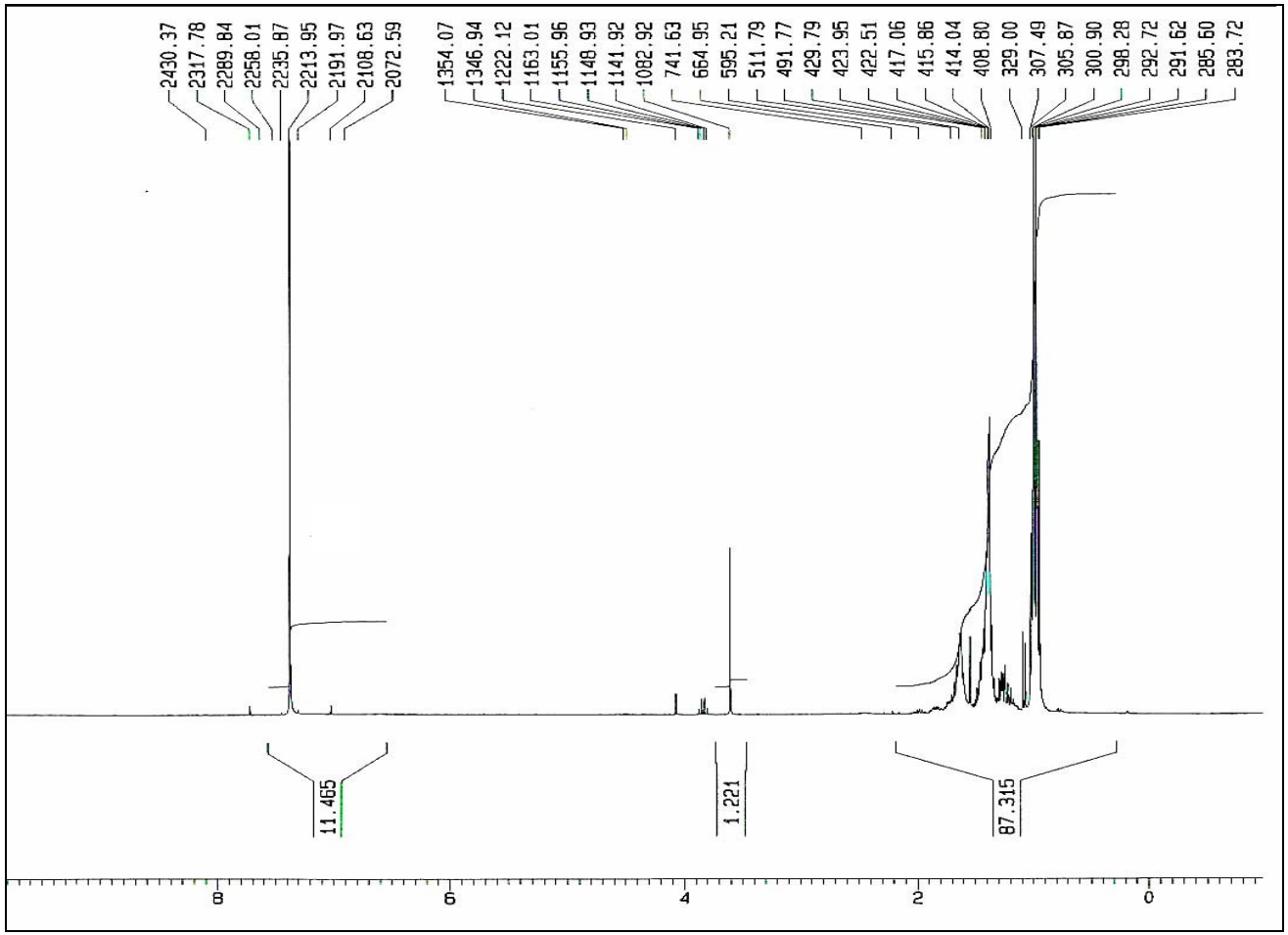


Figure 7.10. The proton spectra from NMR analysis of the twelfth (bota ar12) pure compound.

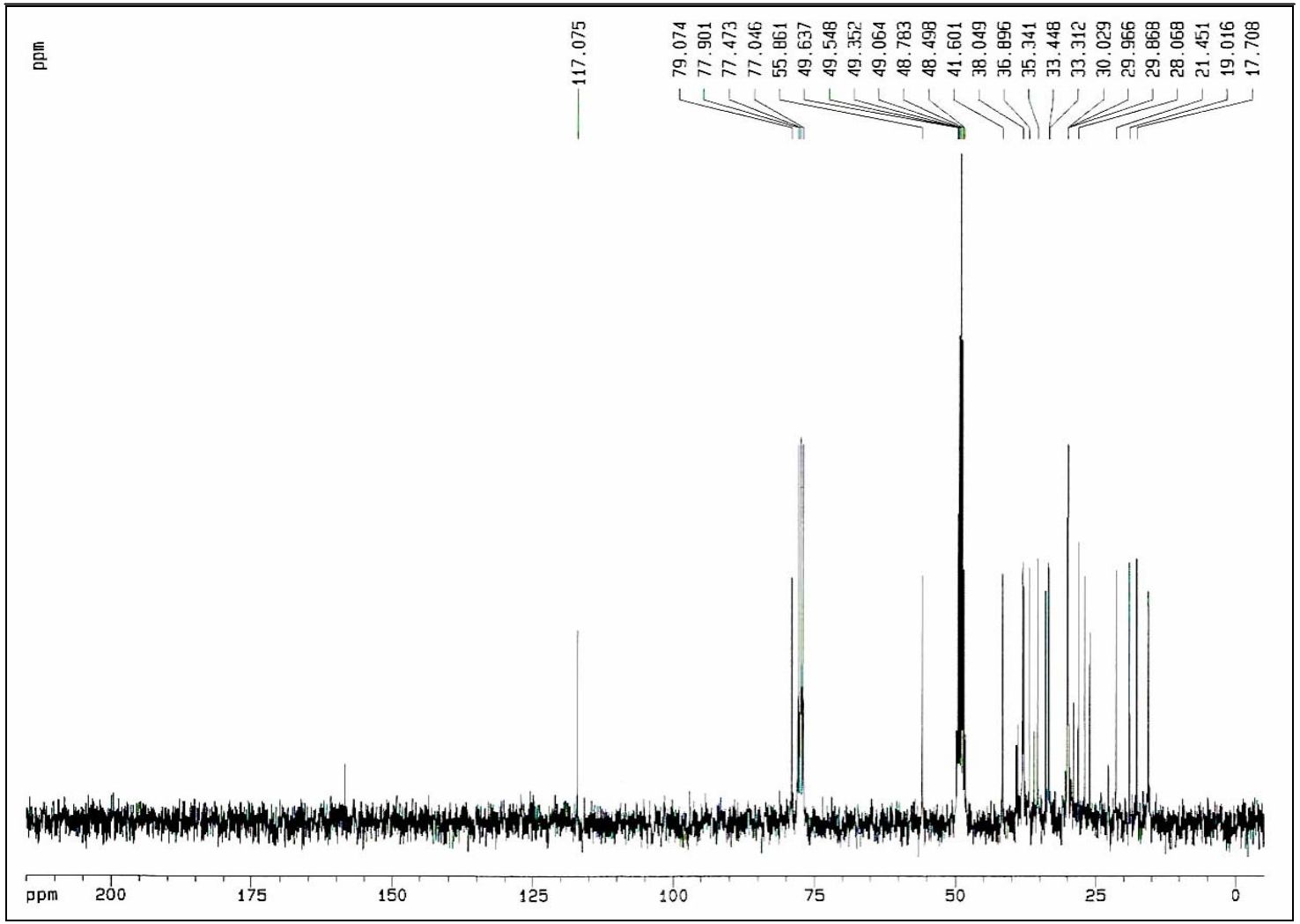


Figure 7.11. The ^{13}C NMR graph of the pure compound bota ar1.

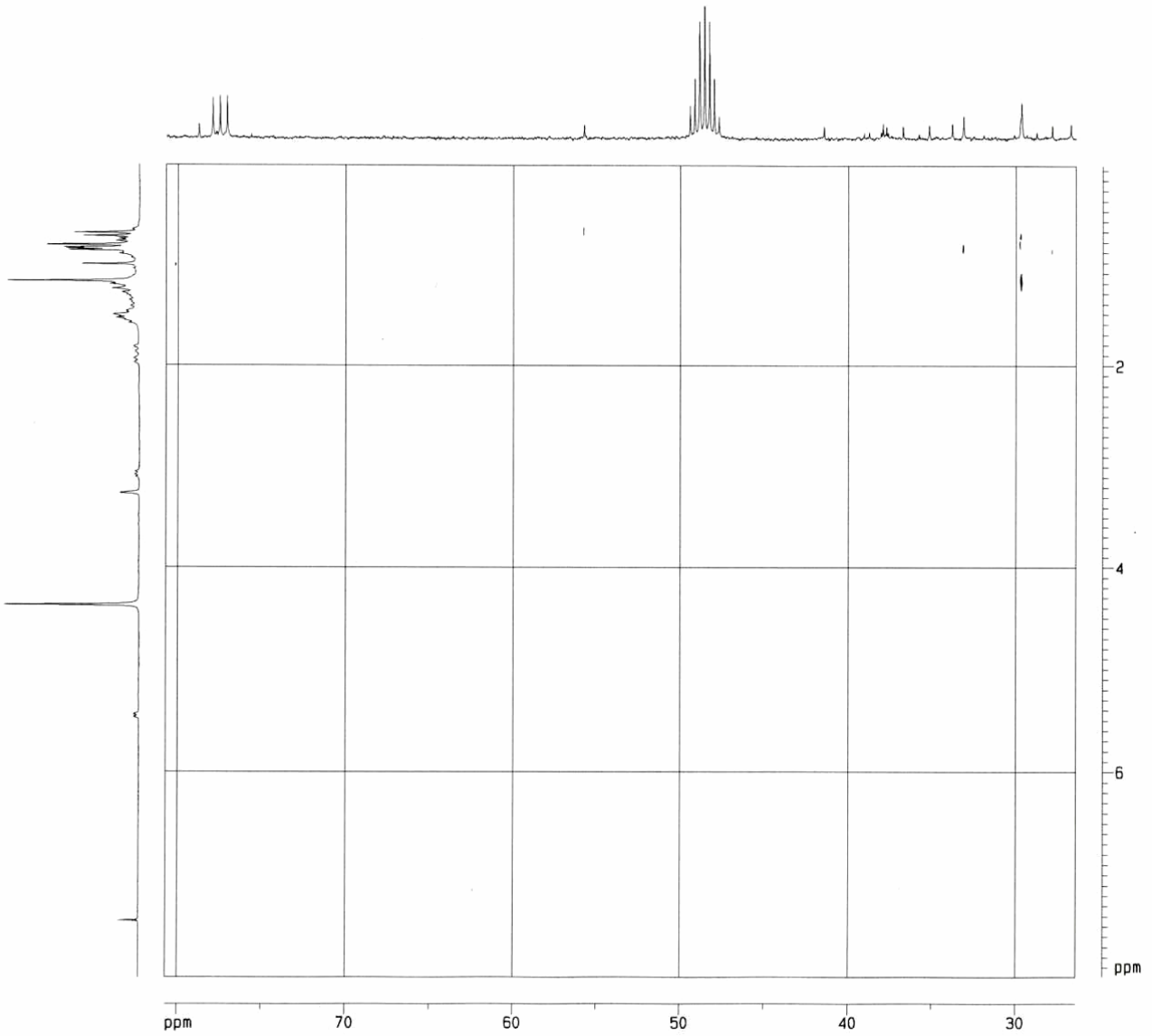


Figure 7.12. The two-dimensional heteronuclear multiple quantum correlation (HMQC) NMR graph of bota ar1.

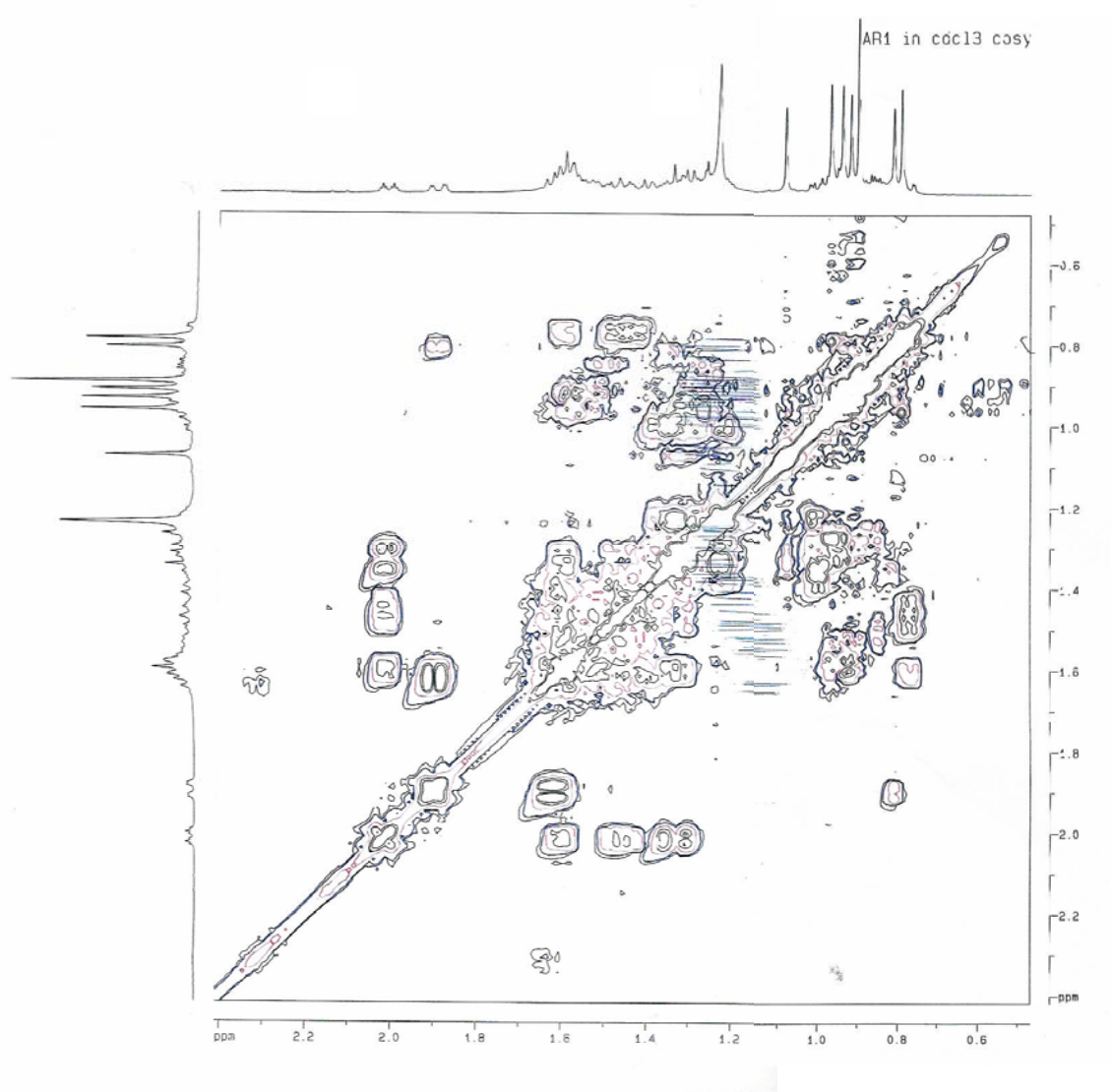


Figure 7.13. The two-dimensional correlated spectroscopy (COSY) NMR spectrum of bota ar1.

When compound bota ar1 dried, it crystallised as colourless needle-like crystals.

Attempts to elucidate the two-dimensional structure of the second compound failed since the material was insufficient (1,8 mg) to obtain the necessary detail.

The 15 other possible pure compounds' structures were not elucidated. They were contaminated by the contents of the deuterated chloroform bottle that was not deuterated chloroform. Efforts to purify these compounds again on Sephadex columns yielded only masses less than 2 mg.

Data from the two-dimensional nuclear magnetic resonance spectra of the first pure compound bota ar1, was compared with similar natural compounds. In Table 7.3, the pure compound isolated (bota ar1) from *P. myrtifolia* leaves, and other similar pentacyclic triterpenes (like taraxerol (2), taraxerone (3), myricadiol (4), and epitaraxerol (5) are listed. It was the same as that of authentic samples of taraxerol, whose details were published in several sources (Sakurai *et al*., (1987), Takasaki *et al*., (1999)).

Table 7.3. ^{13}C NMR values of the pure compound isolated, bota ar1 (1) from *P. myrtifolia* leaves, taraxerol (2), taraxerone (3), myricadiol (4), and epitaraxerol (5). Values for (2) – (5) are from Sakurai *et al*. (1987).

	ppm				
Carbon No.	1 (bota ar1)	2 taraxerol	3 taraxerone	4 myricadol	5 Epitaraxerol
C-I	38.0	38.1	38.4	37.8	37.8
C-2	27.2	27.3	34.1	28	25.2
C-3	79.1	79.2	217.3	78.2	76.3
C-4	39.0	39.1	47.6	41.4	39.1
C-5	55.5	55.7	55.8	56	49.4
C-6	18.8	19.0	20	19.2	18.8
C-7	35.1	35.3	35.2	36.3	33.9
C-8	38.8	38.9	38.9	39.3	38.9

C-9	48.7	48.9	48.7	45.6	48.9
C-10	37.7	37.9	37.6	37.8	37.9
C-11	17.6	17.7	17.5	I7.9	17.5
C-12	35.8	35.9	35.8	31.2	35.2
C-13	37.6	37.9	37.7	38.3	37.9
C-14	158.0	158.1	157.6	158.7	158.1
C-15	116.8	117.0	117.2	116.8	166.6
C-16	36.7	36.9	36.7	33.2	36.8
C-17	38.0	38.1	37.7	38.3	38.1
C-18	49.3	49.4	48.8	49.6	49.1
C-19	41.3	41.4	40.7	41.7	41.4
C-20	29.4	29.0	28.8	28.8	29.0
C-21	33.7	33.9	33.6	33.8	33.2
C-22	33.1	33.2	33.1	28.7	32.4
C-23	28.0	28.1	26.2	28.4	33.1
C-24	15.5	15.6	21.5	16.5	22.0
C.25	14.2	15.6	14.8	15.7	30.0
C-26	31.9	30.1	29.9	30.1	26.5
C-27	25.9	26.0	25.6	26.2	30.0
C-28	29.9	30.1	29.9	64.6	21.2
C-29	33.4	33.5	33.4	33.8	28.1
C-30	21.4	21.5	21.4	22.0	15.1

Bota ar1 gave a molecular ion peak at *m/z* 426.3834 corresponding to the molecular formula $C_{30}H_{50}O$ (calculated 426.3861). The ^{1}H NMR spectrum gave signals for eight tertiary methyl groups: at (δ): 0.98 (H_3-23), 0.80 (H_3-24), 0.93 (H_3-25), 1.09 (H_3-26), 0.95 (H_3-27), 0.82 (H_3-28), 0.91 (H_3-29), 0.91 (H_3-30), 3.19 (dd, *J* = 4.9, 10.9 Hz, H-3α), a typical 3-αH adjacent to 3β-OH and olefinic proton at 5.53 (dd, *J* = 34, 8.0Hz, H-14).

The chemical shifts in the ^{1}H and the ^{13}C NMR spectra of bota ar1 closely resembled those of ^{14}-taraxerene derivatives, particularly the chemical shift and multiplicities of the olefinic proton and the chemical shifts of ring C, D and E carbon atoms. The relative stereochemistry of 3-C, was confirmed by NOE difference measurements. Irradiation at δ3.71 (6- βH) resulted in a 9.12% NOE at δ1.10 (26-H_3), a 7.6% NOE at δ0.97 (25-H_3) and a 10.99% NOE at δ0.90 (24-

H_3). Irradiation at δ0.90 (24-H_3) resulted in a 12.10% NOE at δ3.71 (6-βH), a 15.6% NOE at δ0.97 (25-H_3) and a 6.6% NOE at δ1.4 (2-H_2). Irradiation at δ0.97 (25-H_3) resulted in an 8.25% NOE at δ3.71 (6-βH), a 17.2% NOE al δ1.10 (26-H_3), a 14.1% NOE at δ0.90 (24-H_3) and a 5.2% NOE at δ1.4 (2-H_2). Finally irradiation al δ1.10 (26-H_3) resulted in an 8.9% NOE at δ3.71 (6-βH), a 16.3% NOE at δ0.97 (25-H_3) and a 7.7%NOE at δ1.37 (12-H_2).

The ^{1}H and the ^{13}C NMR data of bota ar1 is the same as data published previously for taraxerol, and is therefore taraxerol. Taraxerol had a melting point of 281 - 282°C (Takasaki *et al*., 1999).

7.3.5 Mass spectrometry

The mass spectrum (Figure 7.14) was characteristic of the ^{14}-taraxerene series of triterpenes. In these molecules a retro-Diels-Alder decomposition would be expected to operate and the collapse of ring D should occur (Indicated in Figure 7.15). The mass spectrum graph of bota ar1 was the same as that of taraxerol.

The mass spectra of members of the □^{14}-Taraxerenes (taraxerone, taraxerol, and myricadiol diacetate) have been measured, thus offering the necessary labels for assigning structures to the major fragments (Budzikiewicz *et al*., 1963). Mass spectrometry can be of great value in the structure elucidation of pentacyclic triterpenes. In general, the presence and position of a nuclear double bond control the fragmentation behaviour, which frequently allow assignment of membership of a given triterpene in one of the major classes (saturated and unsaturated members of the α- and β-amyrin group as well as members of the taraxerol, bauerene, friedelane and lupane series). In addition, the location of functional groups can often be narrowed down by consideration of the fragment pattern (Budzikiewicz *et al*., 1963).

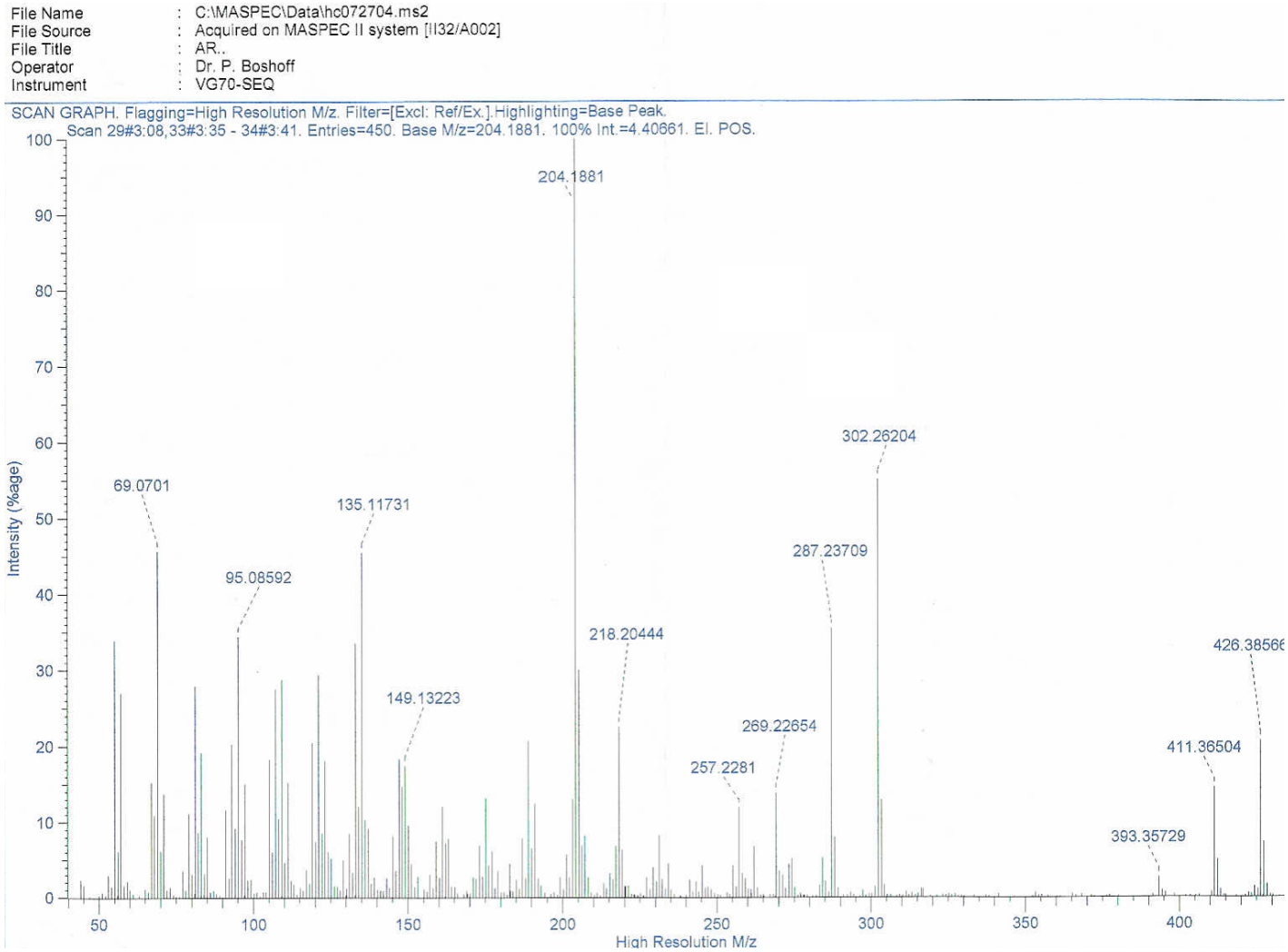


Figure 7.14. The mass spectrum of bota ar 1.

A two-dimensional representation of the structure of taraxerol, with a likely retro-Diels-Alder decomposition which would be expected to operate with collapse of ring D, is presented by Figure 7.15.

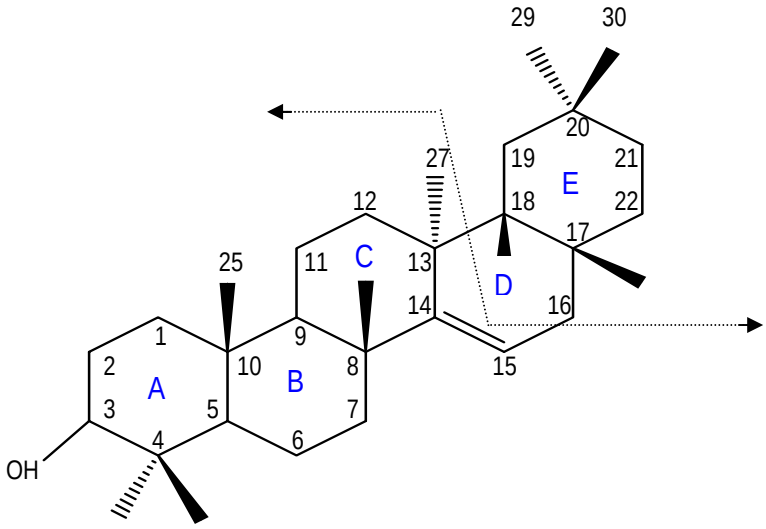


Figure 7.15. A two-dimensional representation of taraxerol. (A retro-Diels-Alder decomposition would be expected to operate with the collapse of ring D).

According to literature sources, the alcohol isolated here, taraxerol, $C_{30}H_{50}O$ was first isolated from *Taraxacum* root, by Burrows & Simpson (1938) who prepared the acetate and benzoate form, but had insufficient material for further study. In 1942, it was isolated from the Japanese *Skimmia* species and largely characterized by Takeda. Although taraxerane derivatives occur in nature in abundance, little study has been done on the parent hydrocarbon in the years before 1962. Confusion arose from literature because Takeda prepared 14α-Taraxerane which he called dihydroskimmiene (Rahman, 1986). In the same year Winterstein assumed Takeda's dihydroskim-miene was oleanane (β-amyrane) and it is so described in Elsevier's Encyclopedia of 1952 (Corbett and Cumming, 1955). Taraxer-14-en-3α-ol was isolated in a previous study in 1962 from the bark of *Suttonia australis.* At that time, it had not been reported to occur naturally, but has since been isolated from *Euphorbia* from Hong Kong and several other plants.

The configuration of rings A and B of 14α-taraxerane is the same as that of ursane, lupane, oleanane and 18α-oleanane (Rahman, 1986).

More recent investigations of taraxerol, indicated that it is identical to skimmiol (Rahman, 1986). 14-Taraxeren-3-ol has a molecular formula of $C_{30}H_{50}O$ and a molecular weight of 426.724. Its synonym is D-Friedoolean-14-en-3-ol. Its appearance is colourless needle-like crystals. The 3α-form is also called Epitaraxerol and the 3β-form Taraxerol or Skimmiol. The 3α-form has been found in *Euphorbia royleana*, *Suttonia australis*, *Macranga denticulate*, *Skimmia wallichii* and other plants. The 3β-form has been found in *Taraxacum officinale* (dandelion), *Alnuss* spp, *Skimmia japonica*, *Rhododendron* spp, *Euphorbia* spp and other plants. It is probably identical with Pertusarin from the lichen *Pertusaria communis*, described by Hesse in 1898 (Takasaki *et al*., 1999).

7.4 Conclusions

Pure compounds were isolated from a bacterially active fraction from *P. myrtifolia* leaves. One pure compound's structure was elucidated as a pentacyclic triterpenoid, taraxerol ($C_{30}H_{50}O$) (synonyms: 14-taraxeren-3-ol, D-friedoolean-14-en-3-ol, or skimmiol). No reports on taraxerol's MIC values for bacteria or tests against human cancer cell lines could be found.

7.5 Literature references

Bisignano G, Germano MP, Nostro A, Sanogo R (1996) Drugs used in Africa as Dyes: II. Antimicrobial activities. Phytotherapy Research 10: 161–163

Budzikiewicz H, Wilson JM, Djerassi C (1963) Mass spectrometry in Structural and Stereochemical Problems. XXXII. Pentacyclic Triterpenes. Journal of the American Chemical Society 85: 3688-3699

Burrows S, Simpson JCE (1938) The Triterpene Group. Part IV. The Triterpene Alcohols of *Taraxacum* Root. Journal of the Chemical Society 1938: 2042 - 2047

Corbett RE, Cumming SD (1972) Lichens and Fungi. Part X1. 14-Taraxerane. Journal of the Chemical Society, Perkin Transactions I, 1135(2): 2827-2829

Cowan MM (1999) Plant products as antimicrobial agents. Clinical Microbiology Reviews 12: 564–582

De Pasquale R, Germano MP, Keita A, Sanogo R, Lauk L (1995) Anti-ulcer activity of *Pteleopsis suberosa*. Journal of Ethnopharmacology 47: 55–58

Elsevier's Encyclopaedia of Organic Chemistry, Vol 14S, Elsevier, Amsterdam, 1952. p. 945S

Famakin JO (2002) Investigation of antibacterial compounds present in *Combretum woodii*. MSc thesis, Pharmacology, University of Pretoria, Pretoria

Grayer RJ, Harborne JB (1994) A survey of antifungal compounds from higher plants (1982–1993). Phytochemistry 37: 19–42

Houghton PJ, Raman A (1998) Chromatographic procedures using columns and liquid-liquid systems. In: Laboratory Handbook for the Fractionation of Natural Extracts. pp. 82-93. Chapman and Hall, London. ISBN 0412749106

Martini ND (2002) The isolation and characterization of antibacterial compounds from *Combretum erythrophyllum* (Burch.) Sond. PhD thesis Pharmacology, University of Pretoria, Pretoria

Ngounou FN, Rahman A, Choudharry MI, Malik S, Zareen S, Ali R, Lontsi D, Sondengam BL (1999) Two saponins from *Pteleopsis hylodendron*. Phytochemistry 52: 917–921

Rahman A (1986) Nuclear Magnetic Resonance, p. 262. Springer, New York

Sakurai N, Yaguchi Y, Inque T (1987) Triterpenoids form *Myrica rubra*. Phytochemistry 26: 217-219

Takasaki M, Konoshima T, Tokuda H, Masuda K, Arai Y, Shiojima K, Ageta H (1999) Anticarcinogenic activity of *Taraxacum* plant. II Biological and Pharmaceutical Bulletin 22(6): 606-610