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BEN-GURION UNIVERSITY
OF THE NEGEV

אוניברסיטת בן־גוריון
בנגב

Beer-Sheva, Israel

באר-שבע, ישראל

BEN-GURION UNIVERSITY OF THE NEGEV
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CHEMICAL STUDIES ON VERNONIA OIL AND GENETIC IMPROVEMENT OF *VERNONIA GALAMENSIS*

Annual report
August 1993 - October 1994

D. Mills, S. Grinberg, J. Chweya and P. Gitu

Submitted to the Office of the Science Advisor
U.S. Agency for International Development
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EXECUTIVE SUMMARY

The aims of the project are to study the chemistry of the very special epoxy oil of the vernonia plant and synthesize novel products with industrial potential; to broaden our germplasm collection; to perform subspecies and varietal trials in a semiarid zone in Kenya and eventually to carry out a commercial trial; and to carry out intraspecific hybridizations and obtain superior plants.

The reporting period includes our work from 1992: we initiated the work before final approval in mid-1993, since there was a delay in the process of approving this program.

We began our investigation of the chemistry of the oil by preparing pure samples of the methyl ester of vernolic acid, which was used as a model compound for bromination (addition and allylic). The bromo derivatives of the methyl ester were synthesized and characterized. Based on these results we performed bromination of vernonia oil with bromine itself and with other bromination reagents. A hexabromo derivative of vernonia oil was obtained as the main product. The brominated vernonia oil will be used in the synthesis of new brominated epoxy resins. Acetylation of vernonia oil followed by oxidation with a permanganate-periodate mixture gave nonanedioic and hexanoic acid.

A trip made recently to two regions in Africa—one in Ethiopia and the other in Kenya—revealed new interesting vernonia germplasm, both in the wild and in populations already collected and in the process of being multiplied. The significance of the populations found in Ethiopia lies in their relatively short growing season, in the timing of flowering (July to August), and in the relatively low water requirement for completion of the life cycle.

In Kenya germplasm collection mission covering vast areas have been undertaken. Fifty three accessions have been collected and are currently being characterized in two places, namely: i) Kibwezi (Dry Land Field Station of University of Nairobi), which is representative of marginal lands, and ii) Muguga (Gene Bank Field), which is situated in an area of high agricultural potential.

Hybridization trials have shown promise, in particular those between two varieties that should contribute to flowering in summer, high seed retention (low shattering), and high yields. Hybrids expressing the latter two traits have not yet been produced, and further trials are being carried out.

During the past year Dr. D. Mills visited Kenya. He participated in a short germplasm collection in the dry Kibwezi area led by Prof. J. Chweya, visited three of the germplasm trials at Kibwezi and Muguga, and helped in the classification and characterization of the plants. He also visited the laboratory of Prof. P. Gitu and discussed the chemistry part of the project. Prof. P. Gitu spent four weeks during August-September in the laboratory of Dr. S. Grinberg in Beer-Sheva. He was accompanied by his student Ms. M. Wawira, who will spend 12 months in the chemistry laboratory in Israel working on the amination of the vernonia oil. The two scientists discussed the program with Dr. S. Grinberg and initiated some experiments.

SECTION I

A) Research Objectives

Our overall objective is to develop *Vernonia galamensis* as a new industrial crop for semiarid zones. To this end, we set ourselves five objectives that we considered would promote the overall aim: 1) to study the chemistry of the very special epoxy oil of the vernonia plant and to synthesize novel products with industrial potential; 2) to broaden our germplasm collection by carrying out expeditions, mainly in Kenya; 3) to perform subspecies and varietal trials in a semiarid zone in Kenya; 4) to eventually perform a commercial trial; and 5) to carry out intraspecific hybridizations to introgress traits from different subspecies and varieties into superior plants.

In the first year of the project we planned to carry out the following activities in Israel: 1) chemical reactions on the double bond of the epoxy oil; 2) selection for self-fertile and self-sterile plants; 3) crossing self-fertile with self-sterile plants selected before the beginning of the project; and 4) growing plants of accession V-001, collecting seeds and extracting oil for the chemical studies. In Kenya we set the following targets: 1) halogenation of vernolic acid; 2) germplasm expedition; 3) growing the germplasm selected and determining parameters such as biomass and seed yield; and 4) growing plants of accession V-001, collecting seeds, and extracting oil for the chemical studies.

B) Research Accomplishments

1. CHEMICAL STUDIES ON VERNONIA OIL

1.1 Chemical work carried out in Israel

1.1.1 Oil extraction and characterization

It was found that Soxhlet extraction yielded somewhat more oil (38%) than stirring ground vernonia seeds with hexane at room temperature (35%). The oil obtained at room temperature contained less free fatty acids, and the oxirane value was higher (3-6%). Other experiments for the extraction of the oil were performed under conditions for lipase deactivation. A method involving lipase deactivation yielded 43-44% of the oil, and as expected under these conditions the oil contained only a small amount of carboxylic groups (0.13-0.33%) and approximately 0.85-2.16% of free fatty acids.

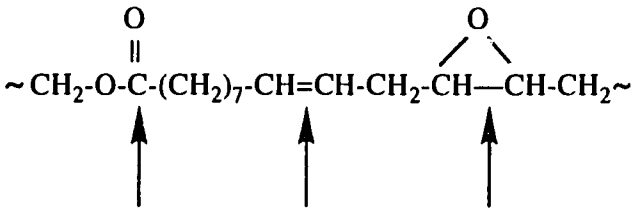
Table 1.1. Fatty acid composition* of *Vernonia galamensis* seed oil (accession V-001 of var. ethiopica, ssp. *galamensis*)

	Soxhlet extraction (%)**	R.T. extraction (%)
Vernolic acid	61-62	79-80
18:2	20	10-12
18:1	5-6	3-4
18:0	3-4	2-3
16:0	3-4	3-4

*As methyl esters by GC/MS.
**The mixture also contains 4-5% of an unidentified product.

A lower content of vernolic acid was obtained by Soxhlet extraction (Table 1.1) (in this case the oil was obtained without lipase deactivation). By extraction of the oil at room temperature the content of vernolic acid was relatively high (80%).

The unique structure of trivernolin enables us to perform a wide variety of reactions characteristic of the ester group, of the double bond, and of the epoxy group (Scheme 1).



Hydrolysis	**Halogenation**	Hydration
Ammonolysis	Isomerization	Alcoholysis
Transesteri-fication	Addition	Amination
	Ozonolysis	Phosphorylation
	Epoxidation	Polymerization
	Hydrogenation	
	Polymerization	

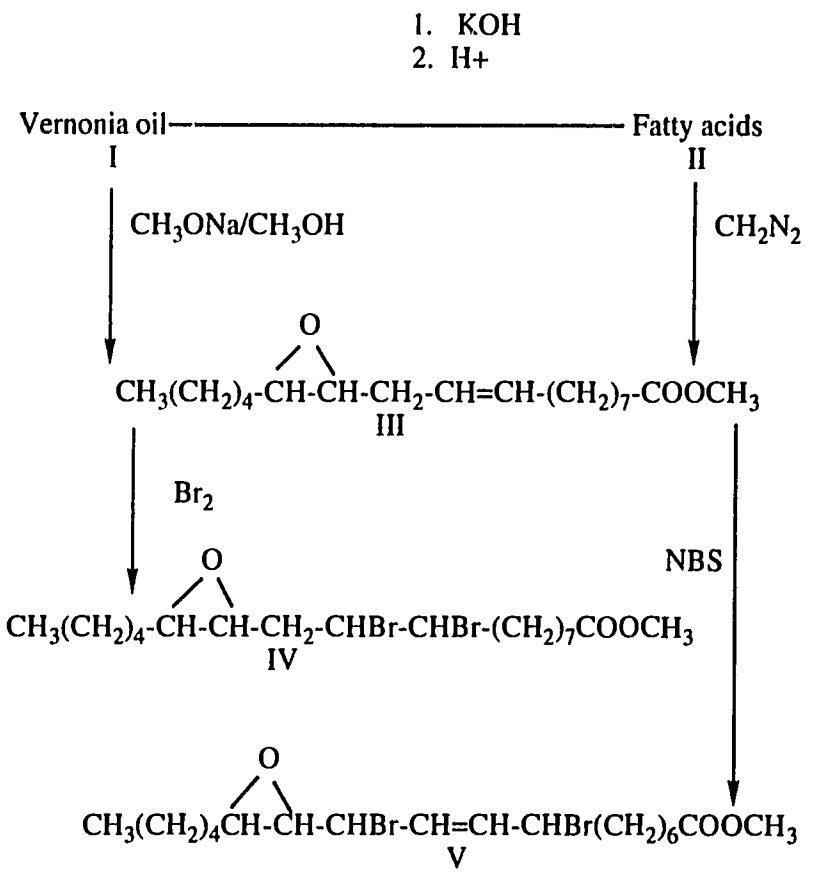
Scheme 1

1.1.2 Bromination reactions

1.1.2a Bromination of the methyl ester of vernolic acid

We began our studies with the halogenation of the double bond, and in this report we describe the bromination of the methyl ester of vernonia oil. The methyl ester was chosen as a model compound that would subsequently enable us to study the chemistry of the more complicated trivernolin molecule with the ultimate aim of preparing brominated vernonia derivatives. These derivatives will find application as intermediates in organic synthesis and as reactive monomers in the plastics industry.

The methyl ester of vernolic acid (III, Scheme 2) was obtained by two methods. The first comprised saponification of the oil (I) with an ethanolic solution of potassium hydroxide; isolation of the fatty acids (II) after acidification [Smith et al. 1959]; and methylation with diazomethane. The relative weight percent of the fatty acids in the oil (I) was determined by GC/MS (Fig. 1.1).



Scheme 2

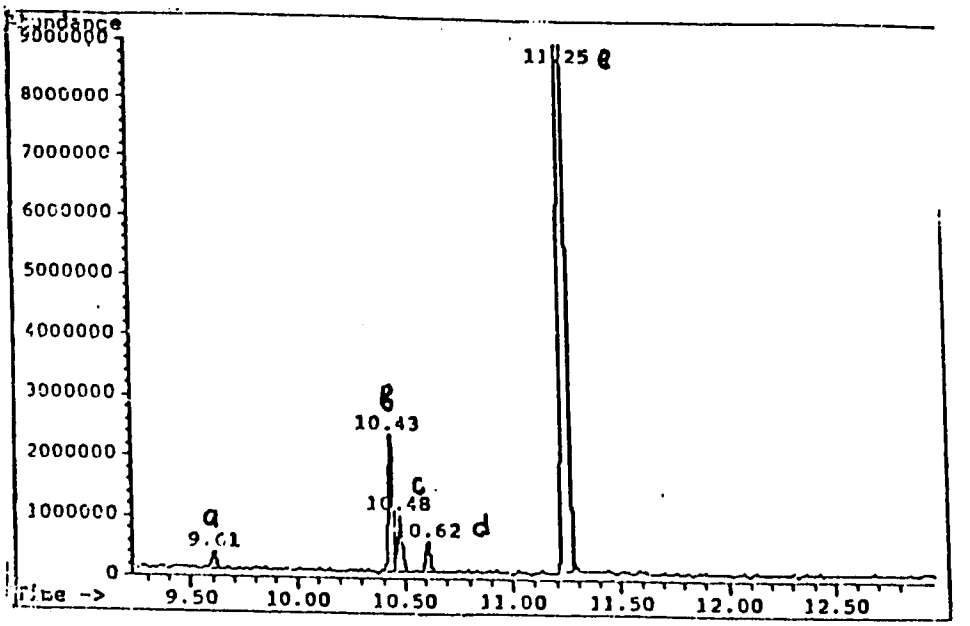


Fig. 1.1. GC/MS of methyl esters of fatty acids of vernonia oil.
Relative wt% of fatty acids: a. palmitic acid - 0.94
b. linoleic acid - 7.44
c. oleic acid - 2.86
d. stearic acid - 1.72
e. vernolic acid - 87.02

The methyl ester of vernolic acid (III) was isolated from the mixture of esters by column chromatography. The spectral data [IR, NMR (Fig. 1.2 & 1.3), mass spectra (Fig. 1.4)] for this sample was completely compatible with the structure III. In the second method of synthesis, the ester III was prepared by methanolysis of the oil (I), followed by isolation of the product by chromatography (Barford et al. 1963). The yields of ester III prepared by these two methods were approximately the same, but the second method is preferable in terms of ease of obtaining preparative amounts.

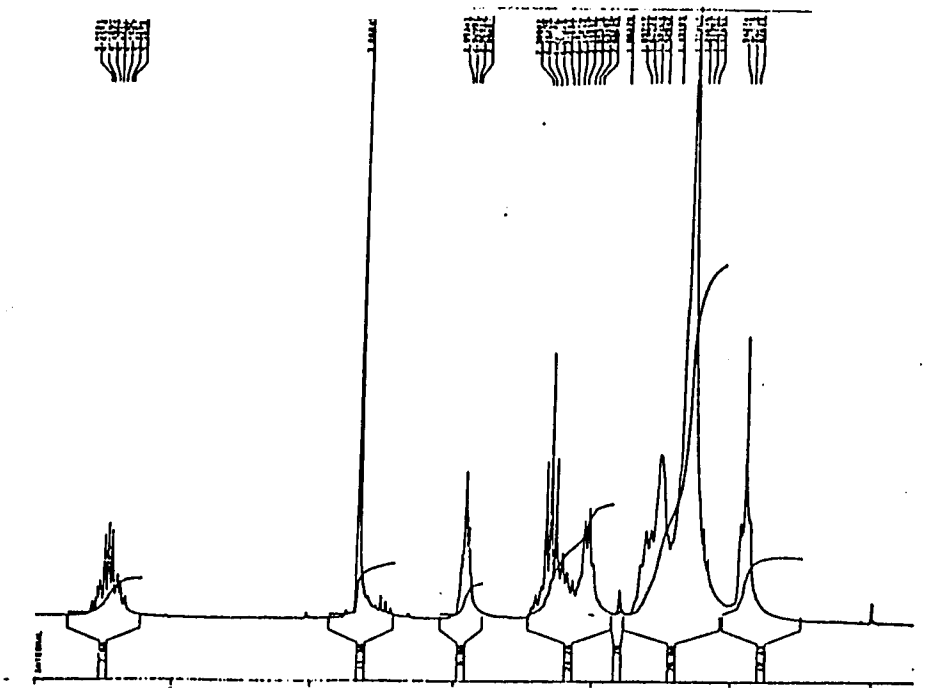


Fig. 1.2 ^{1}H NMR spectrum of methyl ester of vernolic acid

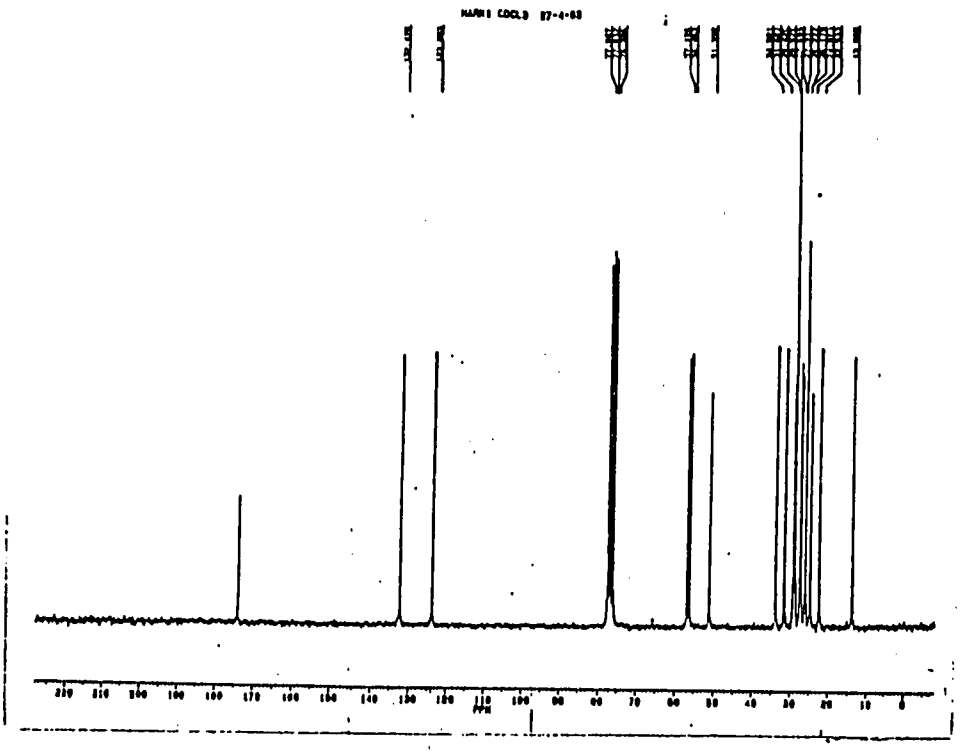


Fig. 1.3 ^{13}C NMR spectrum of methyl ester of vernolic acid

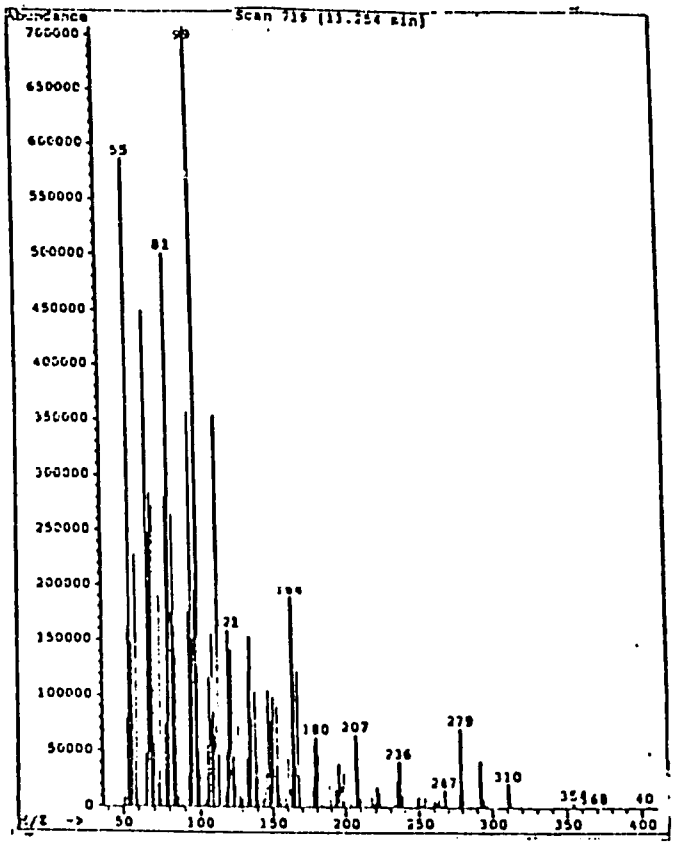


Fig. 1.4. Mass spectrum of methyl ester of vernolic acid

The bromination of the 9,10 double bond of the ester III was carried out at different temperatures in various solvents (carbon tetrachloride, dichloromethane, and acetonitrile). The best results were obtained with carbon tetrachloride at 0°C. A chromatographically pure sample of the dibromine IV was isolated by column chromatography (TLC, GC).

From the ^{1}H-NMR spectrum (Fig. 1.5) it is obvious that addition of bromine to the molecule occurred at the 9,10 double bond. Decoupling of signals at 3.02, 3.16 (epoxy protons) and 4.18, 4.40 (CHBr protons) showed that each pair of protons is vicinal and there was no interaction between these couples. Since it is known that bromine adds *trans* to the double bond, we can expect the ester IV to be a *threo* isomer (a partial structure is shown in Fig. 1.6). However, in the ^{13}C-NMR spectrum (Fig. 1.7) there are low intensity signals (15-20%), which obviously correspond to the *erythro* isomer (partial structure, Fig. 1.6b).

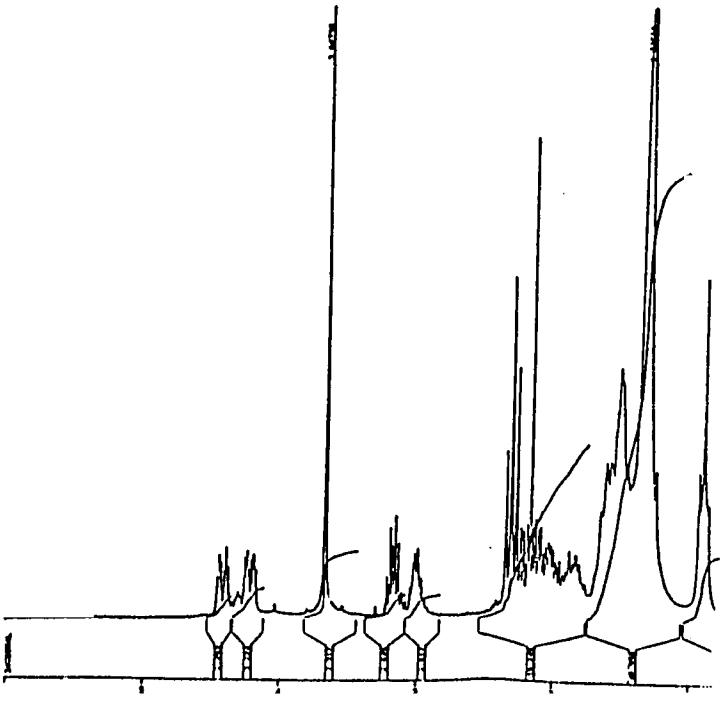


Fig. 1.5. ^{1}H-NMR spectrum of the 9,10-dibromo methyl ester of vernolic acid

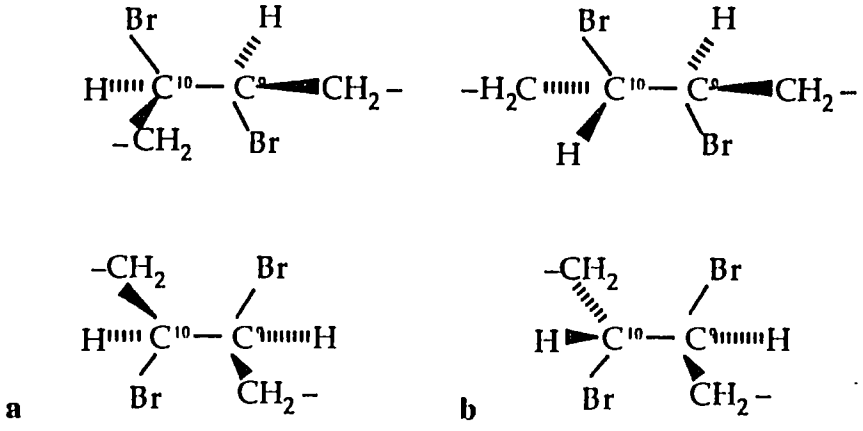


Fig. 1.6. Partial structures of a) *threo* and b) *erythro* isomer of the ester IV

We also carried out bis-allylic bromination of ester III with N-bromosuccinamide (NBS). Interaction of ester III with NBS under different conditions gave a complex mixture of bromo compounds. The product obtained by column chromatography contained only 60% of the main substance. In the ^{1}H-NMR spectrum of this substance there were signals of the epoxy group 2H at 3.02, the CHBr group 2H at 4.49 and 4.67, and olefinic protons 2H at 5.90 ppm (V, Scheme 2).

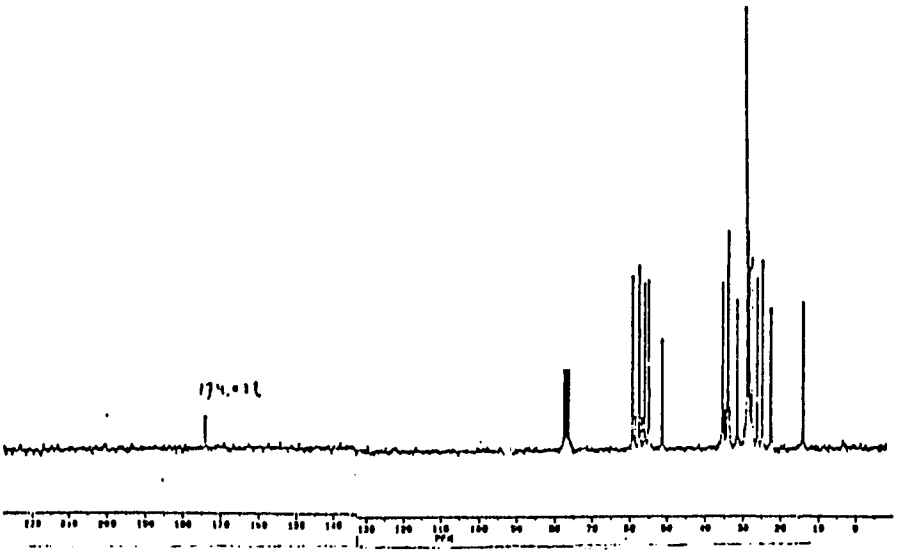


Fig. 1.7. ^{13}C-NMR spectrum of the 9,10-dibromo methyl ester of vernolic acid

1.1.2b Bromination of vernonia oil

Based on the results obtained from the bromination of the methyl ester of vernolic acid, we began a series of bromination reactions of vernonia oil.

The oil that we used for bromination was obtained from the seeds after deactivation of vernonia lipase. It is clear that this oil is different from the oil that we obtained previously, the main difference being that the oil used in this study contained practically no free fatty acids.

The ^{1}H-NMR spectrum (Fig. 1.8) of the oil used in this study showed the presence of an epoxy group at 2.92 ppm (m). The primary protons of the glycerol moiety apparently gave rise to the complex signals at 4.25 ppm, while the secondary proton of a triglyceride glycerol moiety exhibited a chemical shift (about 5.30 ppm) almost identical with that of the olefinic protons (about 5.43 ppm).

In the ^{13}C-NMR spectrum (Fig. 1.9) of the oil signals assigned to vernolyl carbonyl carbons appeared at 173.09 and 172.65 ppm; to vernolyl olefinic carbons at 132.47 and 124.00 ppm; to glycerol carbons at 62.10 (1,3-) and 68.79 (2-) ppm, and to epoxy carbons at 57.09 and 56.50 ppm.

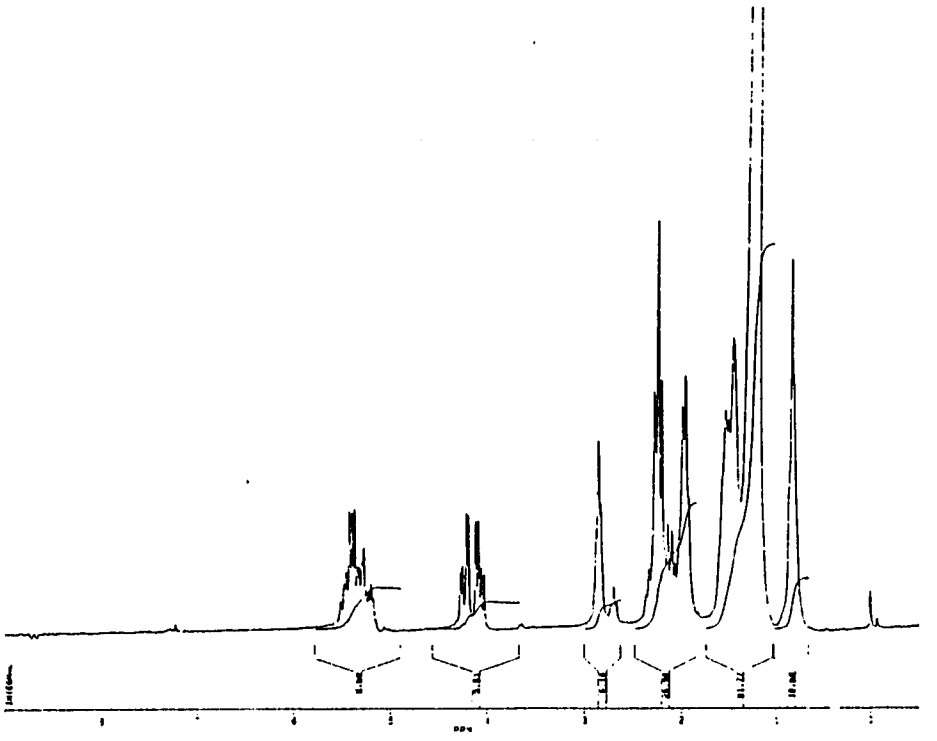


Fig. 1.8 ^{1}H NMR spectrum of vernonia oil

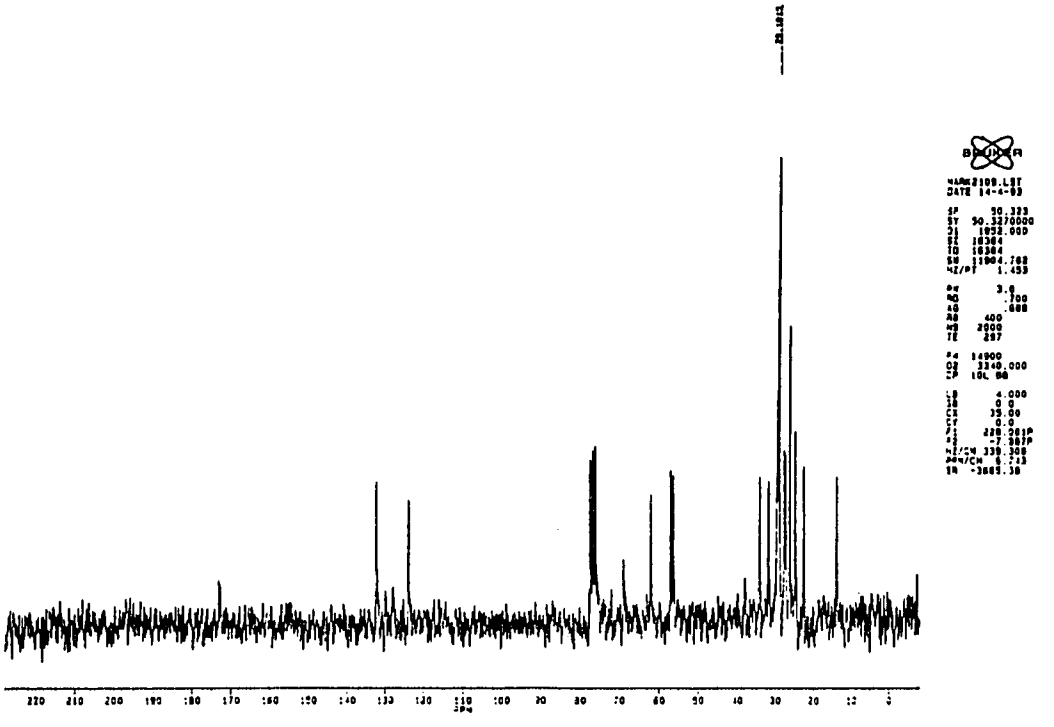
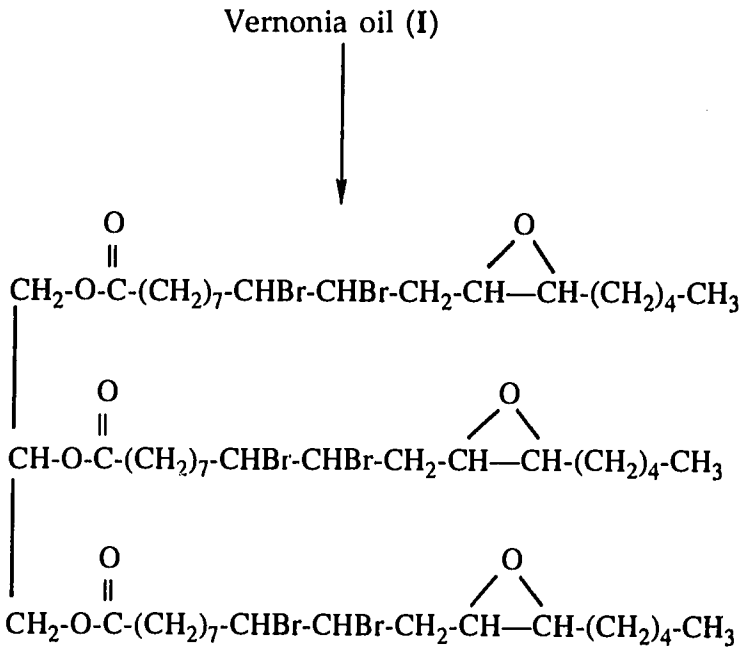


Fig. 1.9. ^{13}C NMR spectrum of vernonia oil

The ^{1}H- and ^{13}C-NMR spectra also confirm that the predominant glyceride is trivernolin.

Bromination of the oil was carried out with bromine, dioxane dibromide ($C_4H_8O_2.Br_2$), and pyridinium hydrobromide perbromide ($C_5H_5NHBr_3$). The reactions were carried out over a range temperatures in different solvents (carbon tetrachloride and dichloromethane). Since the predominant glyceride is trivernolin (I) all calculations were performed on the basis of this compound (Scheme 3).



VII

Scheme 3

From the ^{1}H-NMR spectrum (Fig. 1.10) of bromocompound VII (Scheme 3) it is obvious that bromine added to the 9,10 double bond: signals of olefinic protons are absent, and the signal at 5.26 ppm belongs to the secondary proton of the glycerol moiety. Signals of the protons of CHBr groups overlapping with the signals of primary protons of glycerol moiety give rise to the complex multiple at 4.08-4.57 ppm. We can see that bromination resulted in considerable changes to the signals of the epoxy protons—they appear as two small multiplets at 2.97 and 3.15 ppm. Similar changes were evident in the ^{13}C-NMR spectrum of this compound (Fig. 1.11).

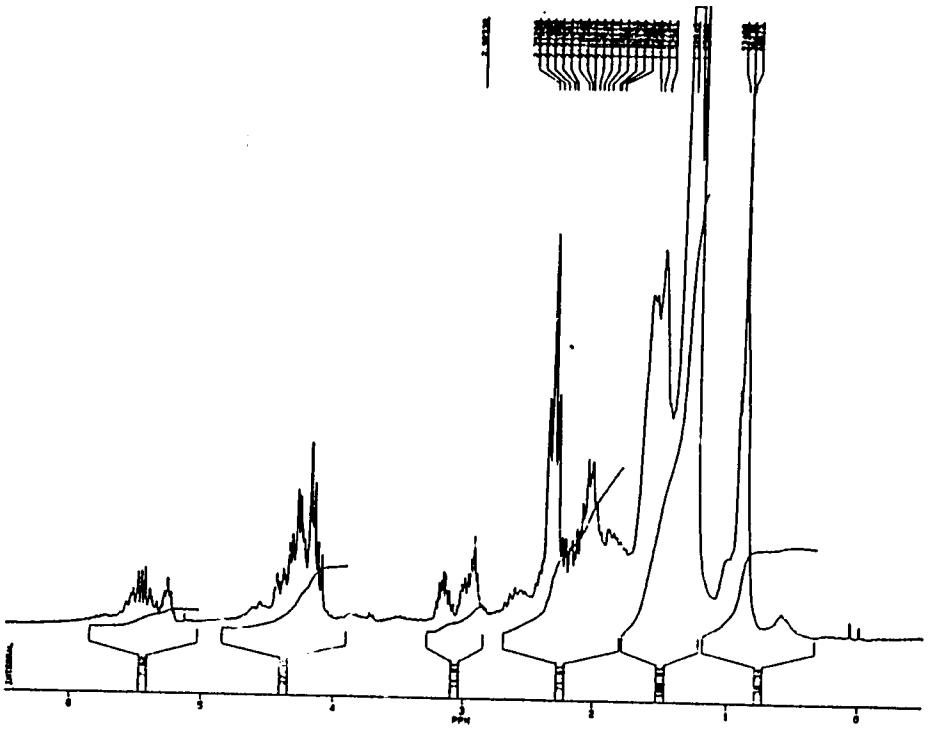


Fig. 1.10 ^{1}H NMR spectrum of the brominated vernonia oil (VII)

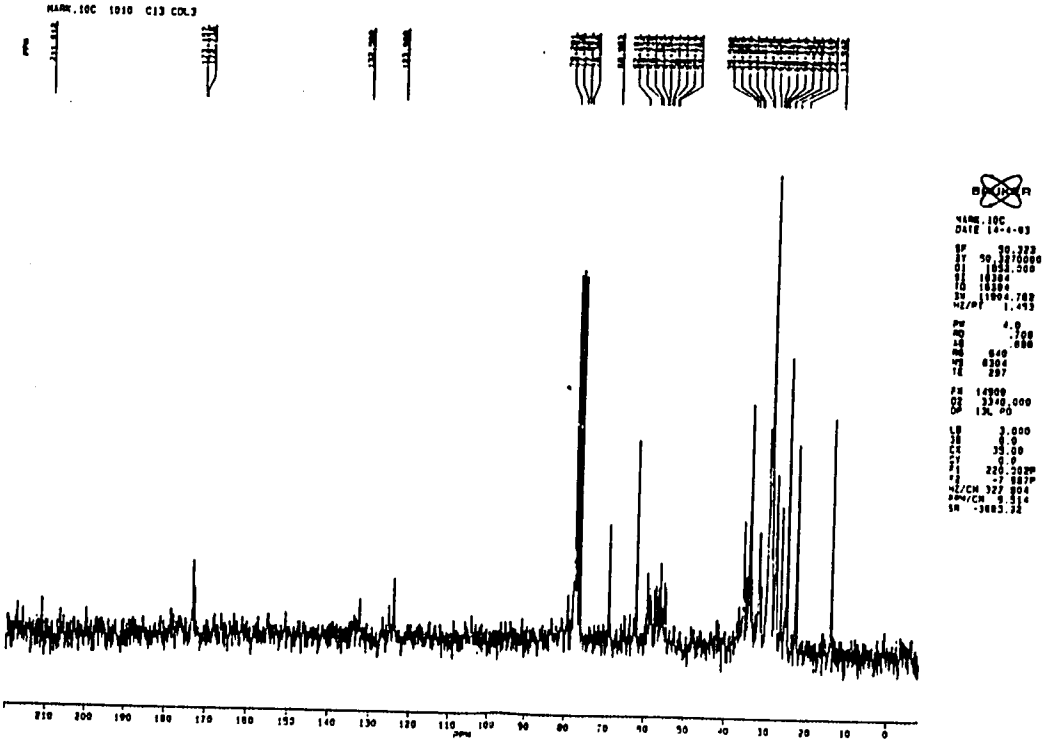


Fig. 1.11 ^{13}C NMR spectrum of the brominated vernonia oil (VII)

It is obvious from the TLC and NMR spectra that in all cases bromination of the oil gave rise to complex mixtures of bromocompounds. We can, however, assume that the structure of the main compound corresponds to the formula VII. It is clear from Table 1.2 that the highest quantity of oxirane groups was obtained when bromination was carried out with pyridinium hydrobromide perbromide, and this is therefore considered to be the method of choice. It is also evident that the oxirane groups in the glycerides are highly labile in bromination reactions in comparison with the methyl ester of vernolic acid.

Table 1.2. Bromination of vernonia oil

Bromination reagent	Yield*	Oxirane groups*	Br (%)*
Br_2	92.4	2.90	35.9
Dioxane dibromide	89.1	3.14	37.2
Pyridinium hydrobromide perbromide	94.0	4.45	35.6

*Isolated, as described in the experimental section
Calcd. for hexabromotrivernolin (VII) $C_{57}H_{48}O_9Br_6$: Br% - 34.14, oxirane groups 8.96%.

For purposes of comparison, we also studied the bromination with bromine of the double bonds of the diamide VIII. (The preparation of this class of compounds will be discussed in the next report.) TLC analyses showed that the interaction of diamide VIII with bromine gave a complex mixture of bromocompounds. On the basis of the ^{1}H-NMR spectrum (Fig. 1.12) seems likely that the structure of the major compound corresponds to formula IX (Scheme 4).

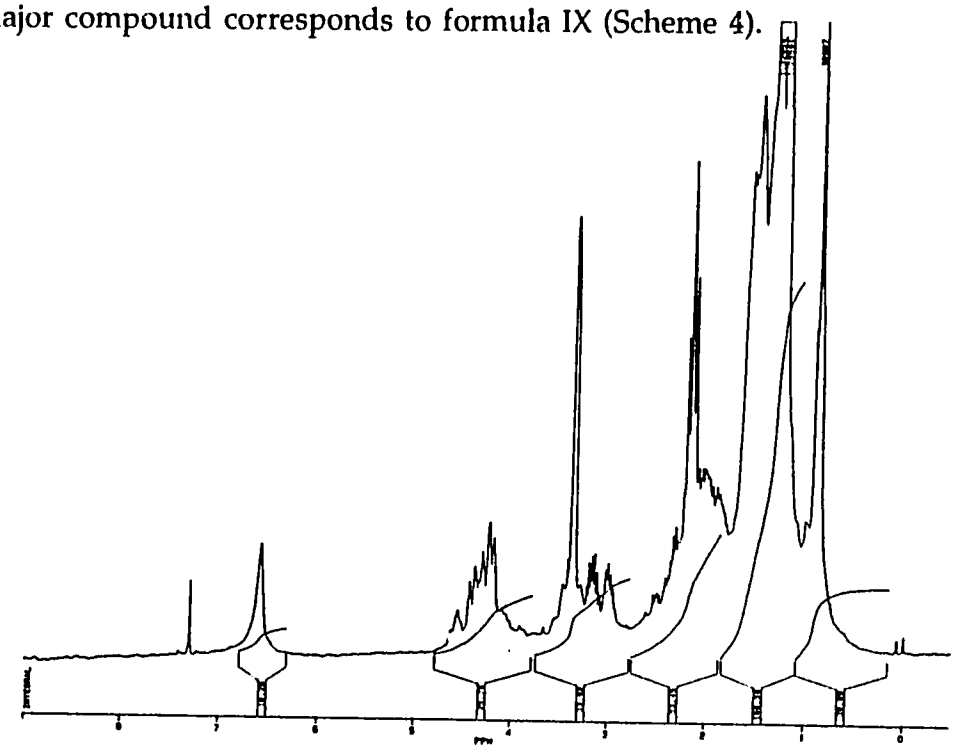
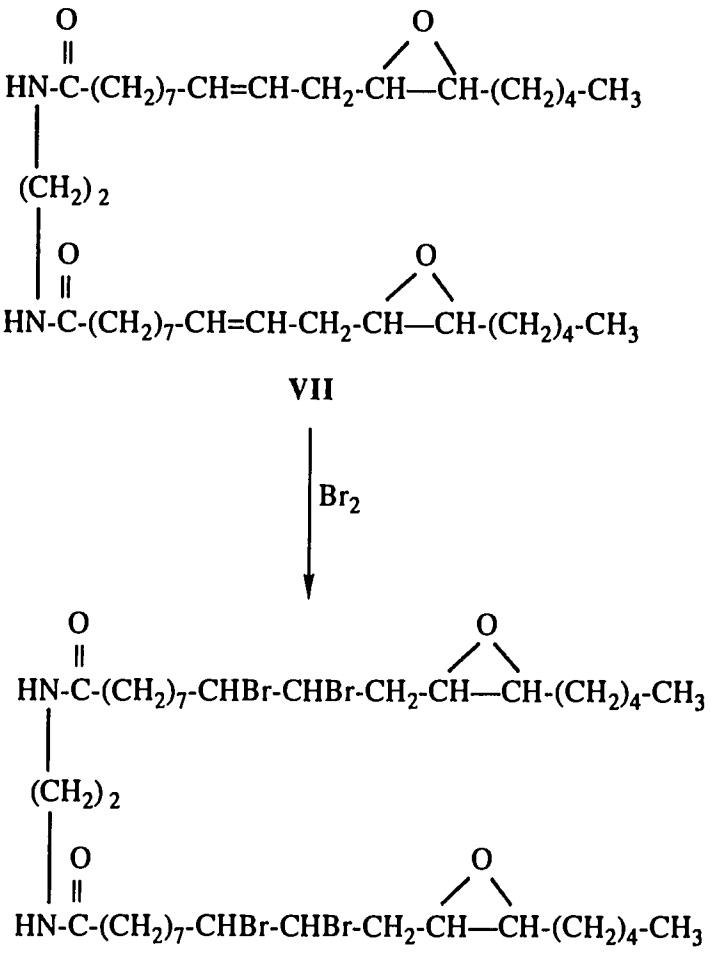


Fig. 1.12. ^{1}H-NMR spectrum of the brominated diepoxy diamide



Scheme 4

1.2 Chemical work carried out in Kenya

1.2.1 Collection, extraction and purification of vernonia oil

1.2.1a Collection of seeds

The seeds were collected from different areas around Nairobi, Kiambu and Embu and dried.

1.2.1b Seed tempering

Tempering of the seeds in order to inactivate the lipase was carried out by three different methods, namely, oven heating, autoclaving and steam inactivation. It was found out that autoclaving and oven heating methods were superior to the steam inactivation method, since a very small amount of free vernolic acid was detected in the oil from the seeds which had ben tempered by these two methods.

1.2.1c Extraction and refining of the oil

After milling of the seeds, the oil was extracted with pre-distilled *n*-hexane. The *n*-hexane solution containing the extracted oil was concentrated in a rotary evaporator *in vacuo* and the crude concentrated oil was refined by heating over activated charcoal followed by filtering. The oil was degummed by stirring with warm distilled water followed by centrifugation. The saponification, iodine and epoxirane numbers and the viscosity of the oil were then determined. A number of trials produced a yield of vernonia oil ranging between 21 and 39%.

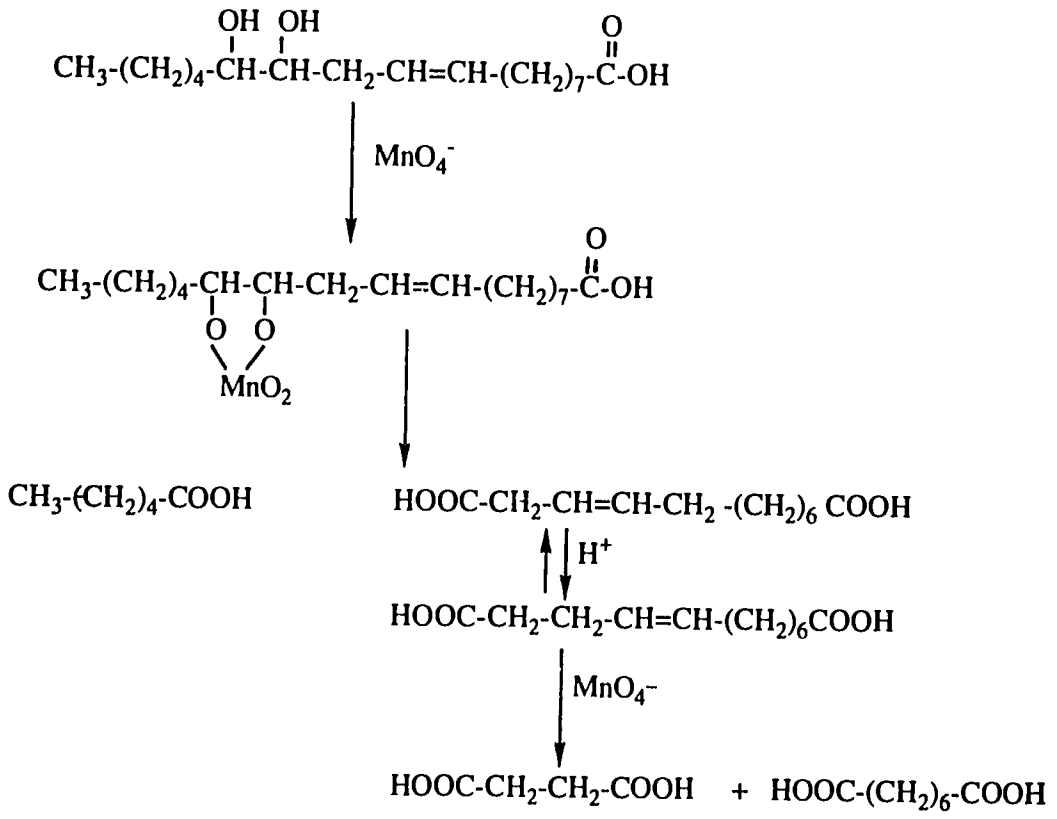
1.2.2. Bromination of vernonia oil

Some reactions involving bromination of vernonia oil were carried out also by the Kenyan team. Isolation and characterization of the products obtained is in progress.

1.2.3 Oxidation of vernonia oil

Vernonia oil was acetylated with acetic anhydride followed by hydrolysis and acidification to give 12,13-dihydroxy-octadec-9-enoic acid in 75% yield. The dihydroxy acid derivative so obtained was oxidized under different conditions to give the following carboxylic acid derivatives:

a) Treatment of 12,13-dihydroxy-octadec-9-enoic acid in an aqueous solution containing *tert*-butyl alcohol with periodate-permanganate reagent at the pH of 8-9 gave nonanedioic acid and hexanoic acid. b) In another oxidation reaction, 12,13-dihydroxyoctadec-9-enoic acid was dissolved in acetic acid and treated with potassium permanganate at 40 to 50°C. After the work up and purification, octanedioic acid, a white crystalline substance with a (m.p. of 140-141°C was isolated and characterized. The EI-MS exhibited the following significant peaks (m/z): 157(50), 152(12), 139(90), 128(10), 115(26), 110 (29), 97(80), 82 (18), 75(55), 69(100), 60(80), 55(95), 45(54) and 41(76). It is likely that this product was formed as result of a carbon-carbon double rearrangement (shifting) followed by oxidation. The postulated scheme is given below (Scheme 5):



Scheme 5

1.3 Experimental

1.3.1 Instrumentation

IR spectra were obtained with a Perkin-Elmer 357 spectrometer, the spectra being run on the samples as such or on samples prepared in Nujol mull. ^{1}H- and ^{13}C-NMR spectra were obtained with Bruker WP-200- SY spectrometer in $CDCl_3$ solution (δ, TMS). Thin-layer chromatography (TLC) analyses were performed on silica gel 60F 254. Solvents were removed under reduced pressure at 35-45°C. GC/MS was performed on a Hewlett Packard 5971A instrument equipped with a capillary column 12 m x 0.2 mm, i.d. 0.33 μ film HP101, temperature program from 70 to 250°C, carrier gas helium. ^{1}H and ^{13}C-NMR spectra were obtained with a Brucker WP-200 SY spectrometer in $CDCl_3$ solution (δ; TMS). TGA analysis was performed on a thermoanalytical system Mettler instrument The epoxy and amino groups were determined by potentiometric titration (Jay 1964).

1.3.2 Oil extraction and characterization

Vernonia oil was obtained by Soxhlet or room temperature extraction of the ground vernonia seeds of accession V-001 of var ethiopica ssp. *galamensis* grown in the field or greenhouse. The seeds were collected and cleaned, and after coarse grinding

the oil was extracted with petroleum ether or hexane. The extraction was performed with or without lipase deactivation (Ayorinde et al. 1990).

Transesterification of Vernonia oil was carried out with sodium methoxide in methanol, and the methyl esters so obtained were analyzed by a GC/MS and compared with fatty acid methyl ester standards. Alternatively, the methyl esters of the free fatty acids were obtained by adding diazomethane solution to the mixture obtained after saponification and acidification.

1.3.3 Methylation of free fatty acids

Approximately 5 mg of the oil was transferred to a 10-ml glass vial, to which was added 1 ml of diazomethane-ether solution. The mixture was shaken thoroughly and allowed to stand for 5 min. A sample of 1.0-2.0 µl was then injected into a gas chromatograph TLC, see fig. 1; GC, see Fig. 3.

1.3.4 Saponification

A mixture of 6.0 g of oil (I, Scheme 2), 1.5 g of potassium hydroxide in 2 ml of water, and 18 ml of 95% ethanol was refluxed for 30 min. The solution was then cooled, diluted with water (100 ml) and extracted with ether (3 x 30 ml) to remove unsaponified matter (solution A). The alkaline liquor was cooled in ice, carefully acidified to pH 5.5-6.0 by dropwise addition of dilute hydrochloric acid, and immediately extracted with ether (3 x 50 ml). The combined ether extracts were dried with magnesium sulfate and the solvent was evaporated off, yielding 3.7 g (61.6%) of mixed fatty acids (II, see Scheme 2; TLC, see Fig. 1). Solution A was also evaporated to give unsaponified matter [yield 0.220 g (3.6%)].

1.3.5 Isolation of the methyl ester of vernolic acid (III, Scheme 2)

To 1.2 g of the mixture of fatty acids (II) was added excess (approximately 10 ml) diazomethane-ether solution, and the reaction mixture was allowed to stand overnight. After removal of the ether 1.25 g of a mixture of methyl esters of fatty acids were obtained (TLC, see Fig 2; GC/MS, see Fig. 4).

The methyl esters were fractionated by column chromatography; the column was eluted with system 1, and fractions of 25 ml were collected. The separation process was monitored by TLC and GC. Fractions 16-22 contained the pure methyl ester of vernolic acid (III). The total yield was 0.75 g. The following analyses were performed:

— TLC (Fig. 2).

— IR (Nujol, cm^{-1}): 1740 (ester carbonyl), 820, 840 (epoxy group).

— Mass-spectrum [Fig. 5 (see Results and Discussion)], m/z: 310 (M^+); 279 (M^+-CH_3O);267 (M^+-$CH_3CH_2CH_2$); 236/M^+-CH_3 $COOCH_3$);
— ^{1}H-NMR spectrum [Fig . 6 (see Results and Discussion)], δ, ppm: 0.91 (3H at C-18, t. J = 6.4Hz), 2.34 (2H at C-2, t. J = 6.2 Hz), 2.93 (2H at C-12 and C-13, epoxy protons, m), 3.66 (3H, OCH_3, s.), 5.47 (2H at C-9 and C-10, olefinic protons, m.)
— ^{13}C-NMR spectrum [Fig. 7 (see Results and Discussion)], δ, ppm: 174.17 (C-1), 132.47 (C-10), 123.89 (C-9) 57.12 (C-12), 56.46 (C-13), 51.35 (OCH_3), 22.53 (C-17), 13.99 (C-18).

1.3.6 Methanolysis of the oil

To 50 ml of a freshly prepared approximately 0.02 N solution of sodium methylate in methanol was added 3.2 g of the oil (I). The reaction mixture was stirred overnight at room temperature. The reaction mixture was then neutralized by adding 0.5 N HCl slowly with stirring. The crude reaction product of the methanolysis was immediately extracted with petroleum ether (b.p. 40-60°C). The extract was washed with water and dried with magnesium sulfate. After removal of the solvent, the residue was fractionated by column chromatography, as described above for the methyl ester of vernolic acid. The total yield of pure methyl ester III was 1.12 g.

1.3.7 Bromination of the methyl ester III to the 9,10-dibromo-ester IV (Scheme 2)

To a solution of 350 mg (1.13 mmol) of the methyl ester of vernolic acid (III) in carbon tetrachloride (10 ml) was added dropwise with stirring and cooling (0°C) a solution of 190 mg (1.2 mmol) of bromine in carbon tetrachloride (10 ml). After 3 h the reaction mixture was washed successively with aqueous sodium bisulfite (3%, 10 ml x 3) and water (20 ml x 3) and then dried with magnesium sulfate. Evaporation of the solvent gave a mixture (470 mg, 86.8%) that contained 79% of the main product (GC). The crude product obtained was subjected to column chromatography (140 g of silica gel), the column being eluted with system 4. Fractions of 25 ml were collected. The separation process was monitored by TLC and GC. Fractions 29-41, containing the dibromoester IV, were collected. These fractions were again subjected to column chromatography (40 g of silica gel), the column being eluted with system 2. Fractions 3-5, each 25 ml, contained chromatographically pure 9,10-dibromoester IV, yield 310 mg. The following analyses were performed:
— IR-spectrum, cm^{-1} : 1730 (ester carbonyl), 840, 810 (epoxy group)
— ^{1}H-NMR-spectrum (Fig. 8), δ, ppm:

0.91 (3H at C-18, t. J = 6.6 Hz), 2.31 (2H at C-2, t. J= 6.7 Hz), 3.02 (H at C-13, epoxy proton m.), 3.16 (H at C-12 epoxy proton m,), 4.18 (H C-9, dt. J = 3.0, 9.4, CHBr), 4.40 (H at C-10, dt. J = 3.1, 9.4, m. CHBr); 2.17 - admixture of acetone.

— ^{13}C-NMR - spectrum (Fig. 9) δ, ppm: 174.02 (C-1), 55.04, 56.07, 57.56, 59.30 (C-9), C-10, C-12, C-13 carbons at epoxy group and bromine atoms, 51.35 (OCH_3),22.49 (C-17), 13.90 (C-18). Signals at 54.71, 56.85, 58.50 ppm (intensity 15-20%) obviously correspond to the erythro isomer. Calcd for $C_{19}H_{34}Br_2O_3$, Br, 33.99%, found 33.60%.

1.3.8 NBS bromination of the methyl ester III to the 8,11-dibromoester V (Scheme 2)

A solution of 400 mg (1.3 mmol) of the methyl ester III and 470 mg (2.6 mmol/of NBS in carbon tetrachloride (15 ml) was refluxed under light (300 W) for 3 h. Filtration of the solid succinimide and evaporation of the solvent gave a complex mixture of bromo compounds (550 mg). The mixture was subjected to column chromatography (140 g of SiO_2), the column being eluted with system 1. Fractions of 25 ml were collected. Combined fractions 15-30 contained 60% of the main compound. The following analyses were performed:

— IR cm^{-1}: 1730 (ester carbonyl), 850, 820 (epoxy group).

— ^{1}H-NMR-spectrum (δ, ppm): 0.91 (3H at C-18, t. J = 6.4Hz), 2.28 (2 H at C-2 t. J = 6.5 Hz), 3.02 (2H at C-12 and C-13, epoxy protons, m), 3.67 (3H, OCH_3, s), 4.49 and 4.67 (2H at C-8 and C-11, CHBr, m), 5.90 (2H at C-9 and C-10, olefinic protons, m). Calc for $C_{19}H_{32}Br_2O_3$, Br 34.2%, found 36.3%.

1.3.9 Bromination of vernonia oil

1.3.9a Bromination with bromine

To a solution of 2.0 g (2.16 mmol) of vernonia oil in 20 ml of carbon tetrachloride, 1.04 g (6.5 mmol) of bromine in carbon tetrachloride (10 ml) was added dropwise with stirring and cooling at 0°C. After 1.5 h the reaction mixture was washed successively with aqueous sodium bisulfite (3%, 15 ml x 3) and water (25 ml x 3) and then dried with magnesium sulfate. Evaporation of the solvent gave 2.80 g (92.4%) of a viscous yellow product. The following analyses were performed:

— IR spectrum: (cm^{-1}) 3550-3450 (hydroxy group); 1760 (ester carbonyl). 860,850 (epoxy group, weak absorption).

— ^{1}H-NMR spectrum, δ, ppm: 0.90 (3 H at C-18, t.J. = 6.04 Ha); 2.31 (2H at C-2, t.J. = 7.2 Hz); 3.15 and 2.97 (weak signal of epoxy protons, m); 4.08-4.57 (protons of

CHBr groups and primary protons of glycerol moiety, m. $w_{1/2}$ = 45 Hz); 5.26 (secondary proton of glycerol moiety, m).

The results of the analysis presented in Table 1.2.

1.3.9b Bromination with dioxane dibromide

To a solution of 2.38 g (2.5 mmol) of vernonia oil in 20 ml of carbon tetrachloride, 2.0 g (8.0 mmol) of dioxane dibromide ($C_4H_8O_2.Br_2$) was added with stirring at room temperature. After 2 h the reaction mixture was washed successively with aqueous sodium bisulfite (3% 20 ml x 4) and water (20 ml x 4) and then dried with magnesium sulfate. Evaporation of the solvent gave 2.870 g (89.1%) of a viscous yellow product. IR spectrum (cm^{-1}): 3560-3470 (hydroxy group); 1740 (ester carbonyl); 830, 845 (epoxy group, weak absorption). The results of the analysis are presented in Table 1.2.

1.3.9c Bromination with pyridium hydrobromide perbromide

A solution of 2.0 g (2.16 mmol) of vernonia oil in 20 ml of carbon tetrachloride was allowed to react with 2.1 g (6.5 mmol) of pyridinium hydrobromide perbromide ($C_5H_5NHBr_3$) with stirring and heating at 40°C. After 6 h a yellow solid was filtered off. The solution was washed successively with aqueous sodium bisulfite (3%, 15 ml x 5) and water (15 ml x 5) and then dried with magnesium sulfate. Evaporation of the solvent gave 2.85 g (94.0%) of a viscous yellow product. The following analyses were performed:

— IR spectrum (cm^{-1}): 3420-3550 (hydroxy group); 1745 (ester carbonyl); 830, 845 (epoxy group, weak absorption).

— ^{1}H-NMR spectrum , δ ppm: 0.90 (3H at, C-18, t. J=6.1 Hz); 2.32 (2H at C-2, t. J = 7.2 Hz); 3.00, 3.16 (protons of epoxy group, m.); 4.10-4.47 (protons of CHBr groups and primary protons of glycerol moiety, m., $w_{1/2}$ = 48 Hz); 5.26 (secondary proton of glycerol moiety, m.).

— ^{13}C-NMR spectrum δ ppm: 173.10, 172.70 (carbonyl carbons); 62.95; 62.09 (glycerol carbons); signals between 59-54 ppm. belong to epoxy carbons and carbons at bromine atoms.

The results of the analysis are given in Table 1.2.

1.3.10 Bromination of the diamide VIII with bromine

A solution of 480 mg (3.0 mmol) of bromine in carbon tetrachloride (10 ml) was added dropwise to a solution of 900 mg (1.5 mmol) of diamide VIII in 1,2-dichloroethane (40 ml) with stirring and cooling (0°C). After 7 h the reaction mixture was washed successively with aqueous sodium bisulfite (3%, 15 ml x 3) and water (20

ml x 3) and then dried with magnesium sulfate. Evaporation of the solvent gave 1.25 g (91.4%) of tetrabromodiamide IX. The following analyses were performed:

— IR spectrum (cm^{-1}): 3380-3450 (NH), 1660 (amide I band), 1550 (amide II band).

— ^{1}H-NMR-spectrum (Fig. 6), δ, ppm: 0.90 (6H at C-18x2, t. J=5 Hz); 2.17 (4H at C-2 x 2 t. J = 7.2 Hz). 3.02, 3.17 (protons of epoxy group, m); 3.37 (NH-$(CH_2)_2$ - NH, 9); 4.38 (CHBr m); 6.49 (NH, s).

Analysis of the product IX, $C_{38}H_{68}Br_4O_4N_2$, gave Br% - 34.3 (cal. 34.1); weight percent of oxirane groups - 5.83 (calc. 8.97).

1.4 Future Plans

- Finding the optimal conditions for bromination of vernonia oil
- Use of the brominated oil for obtaining brominated epoxy resins
- Isolation of 1,3-divernolin
- Bromination of 1,3-divernolin to give a new brominated defunctional monomer.
- reactions with aliphatic and aromatic amines

2. GENETIC IMPROVEMENT OF *VERNONIA GALAMENSIS*

2.1 Materials and Methods

2.1.1 Breeding

A number of subspecies and varieties of ssp. *galamensis* (accessions V-001, V-029, V-018, V-025, V-026, and V-020) were established in 10-liter buckets in two different greenhouses—a glass-covered greenhouse and a plastic-covered greenhouse. Rows were arranged in such way that V-001 was always kept in between two other accessions. When the plants flowered, crosses were performed between var. ethiopica (accession V-001) and the other accessions. Pollination was done manually by rubbing flower heads together. Control pollination was carried out within each accession. After pollination, flowers were tagged and bagged. Bags containing flower heads of each cross were collected after the plants dried up. Seeds in each flower (cross) were counted and recorded, and seeds were pooled. Total weight and one-thousand-seed weight were determined.

2.1.2 Hybrid evaluation

Vernonia plants, hybrids and parents were either sown directly in the field or transplanted after growing in a greenhouse for 5-8 weeks. For direct sowing, seeds were planted at a depth of 2-3 cm in beds with 30 cm between rows and 90-100 cm between beds. Seeds were sown either 30 cm apart or continuously at a density of up to 20 seeds per meter. When good germination was obtained, seedlings were thinned out to a density of 7-8 plants per meter.

For greenhouse sowing, seeds were germinated in petri dishes and incubated in closed humidified boxes in a growth room at 25°C. The seedlings were transplanted into speedling trays 1-2 weeks later and grown in a greenhouse for 4-8 weeks prior to transplanting in the field. Vernonia speedlings were planted 0.5 m apart, and each speedling was placed near a dripper. A drip line with 4-liter-h^{-1} drippers every 50 cm was placed in the middle of the rows in a bed. Irrigation was supplied once a week for seven months up to a total quantity of 900 mm, which was 70% of class A pan evaporation. Fertilization was supplied in the form of compressed pellets containing a mixture of chicken and cow manure enriched with phosphorous and potassium. Weeding was performed by hand within the rows, and between the rows weeds were controlled by careful spraying with Dukatalon (perquat and diquat).

2.2 Vernonia germplasm - activity in Israel

2.2.1 Search for *Vernonia* germplasm in Africa

2.2.1a Kenya

A short exploration trip of four days for vernonia plants was carried out from July 4 to July 7 in the highland in and near Nairobi, along the Nairobi-Nukuro road, and mainly in Kibwezi region from Kiboko river at the Nairobi-Mombasa road (Hunters Lodge) to the town of Voi, covering the main road and some side dirt-roads in the area. Dr. D. Mills was escorted by Mr. N.E. Seme, the Director of the Seed Bank of Kenya, and by Dr. J. Chweya, Head of Crop Science Department, University of Nairobi. This short trip was carried out with the aims of: 1) adding more accessions to those from a similar previous search that was carried out by a Kenyan team during May 1993; 2) classifing the populations in the region since the collaborators from Kenya are as yet inexperienced with the classification of vernonia; and 3) examing and classifing accessions grown in plots for seed multiplication and evaluation.

In Nairobi region, the *V. galamensis* species is dominated by ssp. *nairobensis*. This subspecies was spotted along almost all roads and also in some backyards. It seems to be a very common weed in the area. The different populations appear to be very similar in phenological characteristics, such as leaf, involucre and phyllary dimensions. Canopy shape was, however, not constant: most of the plants were tall and slender, but others were more robust with more side branches. This difference was attributed to soil conditions: it is postulated that moisture, nutrients and a higher portion of sand in the soil (better drainage) stimulate growth of side branches.

Along the roads in the Kibwezi area it was easy to spot quite a few populations of *V. galamensis* spp. *galamensis* var. petitiana. Since the long rainy season finished in April (somewhat sooner than expected because of a drought), most of the plants were dry and had shed their seeds. However, in two locations plants of var. petitiana still flowered, and seeds were collected. These locations were 3 km south of Mitoto Andel on the road to Murka and 5 km north of Makindo on the Nairobi-Mombasa road. In addition to var. petitiana, dry plants of ssp. *mutomoensis* were found 18.3 km north of Voi on the Nairobi-Mombasa road. The seeds collected are in the possession of the Gene Bank of Kenya and will be requested through procedures of the quarantine authority of Israel.

It should be noted that during 1993, the Gene Bank of Kenya made collections from 55 populations from 14 different districts. The different accessions are currently being grown in three locations in Kenya, two of which are in the Kibwezi area, i.e., the

farm of Kenya Agriculture Research Institute (KARI) and the farm of the University of Nairobi. The third location—Muguga (35 km north of Nairobi)—is the site of the Gene Bank itself. It was therefore possible to classify and evaluate all these accessions. In total, four subspecies of *Vernonia* were collected: *galamensis*, *gibbosa*, *nairobensis* and *mutomoensis*. Two varieties of ssp. *galamensis* were collected: petitiana and ethiopica. Basically, populations of these germplasms, with the exception of ethiopica, have been collected in the past from the same areas. The finding of var. ethiopica in the region of Taita Taveta (south west Kenya) was a great surprise, since this variety has been regarded as being indigenous to Ethiopia. Except for inward folding at the tip of the inner phyllaries, the plants seem identical to accession V-001 made by Dr. Perdue in 1984 in Harar, Ethiopia and classified as var. ethiopica by Gilbert (1986). It is yet to be determined if these plants of var. ethiopica were not perhaps "escapees" of a trial carried out in Kenya with seeds collected in Ethiopia.

Some interesting observations were made on the variation in growth and on flowering time between the subspecies and varieties and between the growing locations. Sowing took place in December 1993, March 1994, and April 1994 in Magugu, at the KARI farm, and at the University farm, respectively. In all three locations the first plants to flower were those of var. petitiana. They were followed by ssp. *mutomoensis* and *gibbosa*. The last to flower were ssp. *nairobensis* and var. ethiopica. Interestingly, the sequence of flowering is similar to that recorded in Beer-Sheva. It is believed (Phatak 1989) that in the northern hemisphere most accessions of vernonia flower in response to short days; only a few (like var. petitiana) are day neutral. Since day and night length are similar throughout the year in the locations in which the plots in Kenya are located, day length is not expected to influence flowering.

Flowering in vernonia seems to be a complex phenomenon and is probably influenced by environmental factors such as soil moisture, nutrients, and temperature as well as by the maturation process of the plant. This hypothesis is supported by two observations made in Beer-Sheva: a) plants of var. ethiopica exposed as seedlings to short days and subsequently transplanted into the field developed the first flower about 12 cm above the soil surface, and all branches (6-8) developed below that first flower to a height of 60-100 cm (after 5 months) <u>with no flowers</u>. b) During the same period, seedlings of the same batch that were kept in the greenhouse branched in a later stage (when the first flower had developed at a height of about 80 cm) and developed secondary branches with numerous flowers. It should

be noted that usually vernonia branches after the first apical vegetative bud differentiates into a reproductive bud. The secondary branches grow, produce flowers and then branches again, and so on.

In Mugugu, where plants were exposed to low night temperatures of 5-6°C, the cold appeared to effect plant growth and cause chlorosis. This was most pronounced in var. ethiopica and was also evident to a smaller extent in ssp. *mutomoensis*. Both accessions were collected from warm areas. Var. petitiana, although found in warm areas, was only slightly affected. Spp. *nairobensis* that grows in this region did not exhibit any chlorotic symptoms.

Regarding canopy shape, it was interesting to note that in nature var. petitiana and probably also ssp. *mutomoensis* are tall slender plants that do not branch much well below the first flower. However, both accessions take the shape of a bush (i.e., branching) when cultivated in Kenya (Table 2.1) or in Israel. In var. petitiana and ssp. *mutomoensis* the height of the first flower from the ground is about half of the plant height while in ssp. *ethiopica* and *nairobensis* it is about 4/5 of the plant height.

Table 2.1. Phenological characterization of representative accessions growning at the KARI farm at Kibwezi, Kenya. Characterization was performed on July 6, 1994. Plants were sown on March 4, 1994. Data were collected from 3 plants.

	Subspecies/var. (accession no.):														
	Nairobensis (34516)			Mutomoensis (34498)			Ethiopica (6202)			Petitiana (6169)			Gibbosa (28890)		
CANOPY	Slender						Slender			Bushy			Bushy		
	Branching at top						Branching at base			Branching at base			Branching at base		
PLANT HEIGHT	117			93			107			120			70		
(cm)	80			116			117			132			57		
	66			100			90			85			65		
							90								
Mean	87.7			103.0			101.0			112.3			64.0		
SD	26.4			11.8			13.3			24.4			6.6		
HEIGHT OF															
FIRST FLOWER	82			54			80			64			48		
(cm)	75			54			80			70			37		
	66			52			85			58			50		
							90								
Mean	74.3			53.3			83.8			64.0			45.0		
SD	8.0			1.2			4.8			6.0			7.0		
LEAVES															
Dimensions (cm)	Length	Width	L/W	Length	Width	L/W	Length	Width	L/W	Length	Width	L/W	Length	Width	L/W
	20.8	2.2	9.455	12	2.6	4.62	15	1.5	10	11.5	1.2	9.583	11	4	2.75
	20.5	2.5	8.2	12	2.1	5.71	15	1.6	9.375	14	1.1	12.73	8	3.6	2.222
	23.6	2.4	9.833	11.8	2.5	4.72	16	1.6	10	14	1.2	11.67	11.2	3.5	3.2
	19	2.1	9.048	12	2	6	11.5	1	11.5	14	1.3	10.77	11.5	3.5	3.286
	17	2	8.5	14	2.3	6.09	15	1.4	10.714						
	19.5	2.1	9.286												
	16.2	1.8	9												
	14.5	2	7.25												
Mean	18.9	2.1	8.8	12.4	2.3	5.4	14.5	1.4	10.3	13.4	1.2	11.2	10.4	3.7	2.9
SD	2.9	0.2	0.8	0.9	0.3	0.7	1.7	0.2	0.8	1.3	0.1	1.3	1.6	0.2	0.5
Edge		Serrulate			Serrulate										
Surface		Smooth			Smooth										

Table 2.1 cont.

	Subspecies/var. (accession no.):				
	Nairobensis (34516)	Mutomoensis (34498)	Ethiopica (6202)	Petitiana (6169)	Gibbosa (28890)
FLOWER					
Color	Mauve	White, pale blue at tip	Mauve	Light mauve	Mauve
Involucre Height (cm)	1.7	1.7	2	1	1.3
	1.7	1.7	2	1.1	1.1
	1.7	1.6	2.1	1.1	1.1
	1.6	1.6	2	1	1.3
	1.7	1.6	1.9	1	1.3
	1.5	1.5	1.8	1	1.3
	1.5	1.6		1	1.5
		1.6		1	1.5
		1.5		1	1.5
Mean	1.6	1.6	1.9	1.0	1.3
SD	0.1	0.1	0.1	0.1	0.2
Number per plant	55	n.d.	25	>100	>100
	20		27		
	4		23		
Mean	26.3		25.0		
SD	26.1		2.0		
Remarks			Tip of inner phyllaries bent inwards	Tip of inner phyllaries greener than base	

n.d. - not determined

2.2.1b Ethiopia

A five-day trip was conducted to the Sidamo region in southern Ethiopia. Due to the desire to visit two specific places remote from each other and due to lack of time, the drive was very intensive and the search was conducted only along main roads, not in side roads or in open fields. The trip consisted of the following route: Addis Ababa, Ziway Awasa, Yabello, Arrero, Negele, Bitata, Goba, Asela, Addis Ababa (~1600 km).

The nature of the expedition was to look carefully for vernonia plants on the road margins, to stop occasionally, and ask local people if they were familiar with the plant and the location in which it grows by first showing a picture and later showing a plant sample. Attempts to find out about vernonia from local people started near Agere Mayam (~450 km south of Addis Ababa) met with no success until the first population was spotted on the road margin about 25 km NNE of Yabello. The population was designated ET1 and characterized (Table 2.2). According to local people vernonia grows in other locations in this area, but no other populations were spotted along the road till Yabello. With the aid of local youths, a population of vernonia was found in a graveyard in Yabello (ET2). A search in backyards and at the beginning of the road leaving Yabello for Konsodid not reveal reveal any more vernonia plants. On the way to Negela, only at the village of Arrero and approaching Negele, people recognized the plant but could not point out where it grew, except for one case in which it was impossible to approach the place by the car. In the town of Negele two populations of vernonia were found (ET3 & ET4) about 100 m apart from each other. No other plants were found on the drive from Negele to Addis Ababa.

2.2.1c Characterization of the Sidamo vernonia germplasm

Gilbert (1986) classified the vernonia plant of this region as ssp. *galamensis* var. ethiopica. However, he also mentioned that there were some suggestions of intergrading with var. petitiana, which grows in the South (Kenya and Tanzania) and in the North (North Ethiopia, Eritrea and Sudan). The findings reported here support this suggestion. The characteristics of the four populations are typical neither solely of var. ethiopica nor solely of var. petitiana, but a mixure of the two. The most striking differences between the Sidamo plants and the Harar plants (the populations of var. ethiopica that are represented by accession V-001) lie in the time of the growing season, in flowering and seed set, and in the developmental stages, which are synchronized with the most wet and the dry seasons. In Harar, the long major rainy season is June to September (the shorter rainy season is March-April).

Vernonia grows during the long rainy season, flowers in September and sets seeds by December. In Yabello, the major season (60%) is March to May (the shorter is September-October). Vernonia grows during the long rainy season, flowers during July and sets seeds by September. This difference may have an important impact on the development of vernonia as a new crop in the Northern hemisphere, since the Sidamo plants may prove to be day neutral, i.e., flower in summer under long day conditions. These plants offer another advantage: their life cycle is shorter, since they flower when they are relatively short. This short season growth feature has important agronomic implications for cultivation in other locations, including those in Africa.

The length of the involucre of the Sidamo plants is about 15 mm, a size that is intermediate between the length in var. petitiana and ethiopica (Gilbert 1986, Table 2.2). The tip of the inner phyllaries is darker than the base. This is a characteristic of var. petitiana and not of var. ethiopica, in which the inner phyllaries are uniformly pale green. The color of the florets is mauve, as in var. ethiopica; in var. petitiana it is blue or light blue. It should be noted that the four populations characterized are not identical to one another. This finding is mostly pronounced by the dimensions, surface and margin appearance of the leaves (Table 2.2).

Table 2.2 cont.

	Population			
	ET 1	ET 2	ET 3	ET 4
LEAVES				
Leaf edge	Serrate	Serrulate	Serrulate	Serrate
Teeth depth (mm)	1	<1	<1	1.5
Surface	Rough	Rough	Smooth	Smooth
FLOWERS				
Flowering status	Flowers on secondary branches	Developed buds just before flowering	Flowers on secondary branches	Flowers on secondary branches
Color	Mauve	-	Mauve	Mauve
Involucre Length (cm)	1.5		1.3	1.4
	1.5		1.4	1.4
	1.6		1.4	1.3
	1.5		1.5	1.5
	1.5		1.5	1.5
	1.6		1.4	1.3
			1.3	1.4
			1.4	1.4
Mean	1.5		1.4	1.4
SD	0.1		0.1	0.1
Inner phyllaries width (mm)	1.5	1.5	1.5	1.5
	Tip greener than base	Tip greener than base	Tip greener than base	Tip greener than base
Outer phyllaries	Tip bent inwards !			
FRUIT	First flowers after anthesis; seeds not ripe	No anthesis	First flowers after anthesis; seeds not ripe	First flowers after anthesis; seeds not ripe

n.d. - not determined

Table 2.2 Phenological characteristics of four vernonia populations found in the Sidamo region, Ethiopia.

	Population											
	ET 1			ET 2			ET 3			ET 4		
DATE	25/6/94			25/6/94			27/6/94			27/6/94		
SITE	25 km NNE of Yabello			Yabello			Negele			Negele		
PLANT HEIGHT (cm)	60-100			30-120			60-70			80-100		
BRANCHING	Yes			No			Yes			No		
LENGTH OF SECONDARY BRANCHES (cm)	n.d.			n.d.			27			n.d.		
							21					
							12					
							20					
							12					
							17					
							17					
							16					
Mean							17.8					
SD							4.9					
LEAVES												
Dimensions (cm)	Length	Width	L/W	Length	Width	L/W	Length	Width	L/W	Length	Width	L/W
	11.5	1.5	7.6667	18	2	9.0	15.3	2.3	6.7	14	2.7	5.2
	11.5	1.5	7.6667	17	1.8	9.4	16.5	2.2	7.5	16	2.7	5.9
	11.5	1.1	10.455	14	1.3	10.8	15.3	2.1	7.3	16.5	3	5.5
	13	1.3	10	13	1.2	10.8	17	2.1	8.1	16.3	2.8	5.8
				12	0.9	13.3	11.5	2	5.8	16.5	2.6	6.3
							13	2.1	6.2			
							13	2.1	6.2			
							12	2.4	5.0			
							15	2.4	6.3			
Mean	11.9	1.4	8.9	14.8	1.4	10.7	14.3	2.2	6.5	15.9	2.8	5.8
SD	0.8	0.2	1.5	2.6	0.5	1.7	2.0	0.1	1.0	1.1	0.2	0.4

2.2.2 Germplasm evaluation

During the period March-May 1993 the following accessions of vernonia were transplanted or sowed in a small plot: V-001 (var. ethiopica), V-004 (var. galamensis), V-009 (var. australis), V-018, V-019, V-025 (ssp. *mutomoensis*), and V-029 (var. petitiana). Emphasis was placed on accessions that had not been tested in previous years. Transplanting was essential for some accessions because of poor germination following direct seeding in the field. For purposes of comparison, accession V-001 was also transplanted. The accessions and numbers of plants established from each are listed in Table 2.3.

All accessions flowered during summer, with the exception of V-001 (Table 2.4). V-029 was the earliest accession to flower (mid-May). Accession V-004 flowered two weeks later, followed by V-018, and then all the other accessions. With the exception of V-001, ssp. *galamensis* requires fewer short days for flowering than other subspecies. V-029 therefore shows promise, since it flowers under conditions of long days. This trait was also exhibited in the two-week difference in flowering between plants sown at a difference of four weeks.

In general, all accessions produced seeds in the field—as opposed to the high degree of sterility that was exhibited in the greenhouse as a result of the absence of insects. It should be emphasized that all our accessions, with the exception of V-001 are not self-fertile. In the field, plants did not produce high yields with the exception of V-004 (Table 2.3). One-thousand-seed weight varied from 2 to 4 g, being low in V-032, V-029, and V009 and high in V-020. It should be also mentioned that in another observation, an increase in the amount of insects, by adding bee hives near the plot, resulted in a heigher seed yield of V-004 plants.

Table 2.3. Germination and flowering of various subspecies, varieties and hybrids grown in the field in 1993

Subspecies	Variety	Accession	Field sowing date	Field transplan-ting date	Germination date	Flowering date
galamensis	ethiopica	V-001	March 3		March 11	None
				March 30*		None
				May 9**		None
galamensis	petitiana	V-029	March 3		April 12	May 15
				March 30*		June 8
				May 9**		June 26
F1 hybrid		V029 X V001	-	March 30		May 15
galamensis	petitiana	V-032	-	April 1		
galamensis	galamensis	V-004	March 3	-	March 23	June 1
galamensis	australis	V-009	March 3	-	March 30	July 2
mutomoensis	-	v-018	March 3	-	March 23	June 10
mutomoensis	-	V-019	March 3	-	March 23	July 2
mutomoensis	-	V-025	March 3	-	March 23	June 27
F1 hybrid		V025 X V001	-	March 30		June 3
afromontana	-	V-020	March 3	-	March 23	June 27

* Sown in a petri dish on January 24 and transplanted to a speedling tray on February 10

** Sown in a petri dish on February 23 and transplanted to a speedling tray on March 11

Table 2.4. Seed yield and one-thousand-seed weight of various subspecies, varieties and hybrids grown in the field in 1993

Subspecies	Variety	Accession	Field sowing date	Field transpla-nting date	Yield/ plant	1000 seed wt
galamensis	ethiopica	V-001	March 3		None	None***
				March 30*	None	None
				May 9**	None	None
galamensis	petitiana	V-029	March 3		5.8	2.6±0.1
				March 30*	1.8	
				May 9**	2.5	
F1 hybrid		V029 X V001	-	March 30	2.7****	3.5±0.3
galamensis	petitiana	V-032	-	April 1	0.4	2
galamensis	galamensis	V-004	March 3	-	32	3.3 ±0.2
galamensis	australis	V-009	March 3	-	None	2.8
mutomoensis	-	V-018	March 3	-	7.6	3.6
mutomoensis	-	V-019	March 3	-	3.4	3.5±0.1
mutomoensis	-	V-025	March 3	-	0.6	3.3
F1 hybrid		V025 X V001	-	March 30	1.6	3.3
afromontana	-	V-020	March 3	-	0.9	3.9±0.4

* Sown in a petri dish in January 24 and transplanted to a speedling tray on February 10.

** Sown in a petri dish in February 23 and transplanted to speedling tray on March 11.

*** One-thousand-seed weight of other lots of V-001 is 3.9±0.1 g.

**** Partial yield.

Accessions of ssp. *mutomoensis* (VA-018, V-019 and V-025), and *afromontana* (V-020) were more robust than ssp. *galamensis* (V-001, V-0045, V-029, and V-032), although accession V-009 was an exception. In general, the former subspecies had less flower heads and less seeds per plant. V-029 is a short plant and did not grow vigorously under the conditions of the Northern Negev. In contrast to the latter,

V-004 is a vigorous plant with many tertiary flowering branches and many flower heads.

Since V-004 flowers in summer and has a relatively satisfactory yield, it is at present considered the best germplasm under our conditions. Nevertheless, it possesses a medium degree of seed shattering - an inferior characteristic that prevents its cultivation.

2.2.3 Hybridization trials

2.2.3a Characterization of hybrid plants of ssp. *mutomoensis* and var. petitiana with var. ethiopica

Samples of F1 hybrid seeds, prepared in 1991 by crossing both ssp. *mutomoensis* (V-025) and ssp. *galamensis* var. petitiana (V-029) with ssp. *galamensis* var. ethiopica (V-001), were either sown directly in the field or germinated in petri dishes and then transplanted into speedling trays and finally into the field. Phenological characteristics of the hybrids and their parents are presented in Table 2.5. It was observed that V-029 x V-001 hybrids grew much more vigorously and were taller than their parents. The other features of the hybrids were intermediate between their parents—height (from soil surface) of first flower (in V-001 this flower is quite high), dimensions of the flower (flower size of V-001 is bigger) and seed weight (see also Table 2.4). The leaf dimensions and the color of the flower resembled those of V-001. Flowering date and, disappointingly, also seed retention resembled V-029. In contrast to the latter, as far as we can tell, the hybrid of V-025 x V-001 did not exhibit intermediate features of its parents, although some parameters were different from those of V-025.

Flower heads of V-029 x V-001 hybrids were bagged in the field and seeds were harvested for assessment of the characteristics of the F2 plants in summer 1994. F2 plants of V-029 x V-001 hybrids were less vigorous and smaller in dimensions compared to F1 plants (see Table 2.9 and Fig 2.1). It is important to note that F2 were less vigorous than their own F1 mother plants; The F2 plants were smaller in dimensions (compare data in Table 2.5 to the data in Table 2.9), and interestingly the length of the leaf was also smaller, deviating from the leaf length of accession V-001 as opposed to the mother F1 plants that had similar leaves to V-001. In addition, the F2 plants flowered (first flower) much later after transplanting (109 days) than the F1 plants (36 days). This was also reflected in the height of the first flower from

the ground—37 and 60 cm for F1 and F2, respectively. The flower color of F2 plants was light blue while F1 plants had a purple (mauve) color.

Table 2.5. Phenological characteristics of vernonia accessions and hybrids grown in the field in 1993

Phenological parameter	V-029		V-001		V029 X V001		
	Sown	Trans-planted	1	2	1	2	3
Height (cm)	65	59	72	63	97	90	85
Width (cm)	45	75	49	42	85	60	40
Height of first flower (cm)	22	8	-	-	36	55	23
No. of branches below 1st flower	12	8	-	-	14	30	15
Length of 2ndary branches (cm)	36.1	28.6			56.8	36.8	61.7
SD	3.7	5.9			9.3	8.6	17.7
No. of 2ndary branches	5.4	5.1			7.3	3.5	7.0
SD	1.2	1.6			1.8	1.0	2.8
Main stem diameter (cm)	1.83	1.55	1.55	1.50	1.90	1.45	1.55
2nd branch diameter (cm)	0.61	0.51	0.46	0.50	0.82	0.58	0.62
SD	0.14	0.04	0.06	0.11	0.20	0.28	0.13
Leaf width (cm)	1.2	1.2	1.2	1.2	1.1	0.9	0.9
SD	0.2	0.1	0.0	0.1	0.1	0.2	0.2
Leaf length (cm)	10.4	7.5	15.4	15.0	15.5	14.6	12.9
SD	1.0	1.5	0.5	2.2	1.2	1.8	1.8
Width/length	0.1	0.2	0.1	0.1	0.1	0.1	0.1
SD	0.01	0.03	0.00	0.02	0.01	0.01	0.01
Flower width (cm)	0.78	0.78			1.29	1.23	1.07
SD	0.07	0.04			0.22	0.01	0.08
Flower length (cm)	1.10	1.06			1.52	1.50	1.62
SD	0.07	0.09			0.08	0.00	0.13
Width/length	0.71	0.74			0.85	0.82	0.67
SD	0.06	0.08			0.14	0.01	0.06
Flower color *	2	1			3	3	3
Distance between flower and closest leaf (cm)	0.37	0.42					
SD	0.22	0.16					

* 0 - white; 1 - light blue; 2 - blue; 3 - purple; 4 - dark purple

Table 2.5. Cont.

Phenological parameter	V-025 1	V-025 2	V-025 3	V-001 1	V-001 2	V-001 3	V-001 4	V-025 X V-001 1	V-025 X V-001 2	V-025 X V-001 3
Height (cm)	162	140	124	120	124	126	131	80	118	93
Width (cm)	64	70	62	20	40	35	25	76	55	60
Height of first flower (cm)	162	138	78					72	98	93
No. of branches below 1st flower	75	100	84					23	52	37
length of 2ndary branches (cm)	63.8	62.0	74.5					62.7	42.3	46.0
SD	3.3	19.8	4.2					9.0	8.2	7.5
No. of 2ndary branches	0.0	0.0	0.0					0.0		0.0
SD	0.0	0.0	0.0					0.0		0.0
Main stem diameter (cm)	2.00	2.20	2.80	1.20	1.70	1.30	1.00	2.30	2.00	1.70
2ndary branch diameter (cm)	0.70	0.55	0.88		0.50	0.36	0.37	0.90	0.55	0.53
SD	0.00	0.13	0.10		0.10	0.09	0.12	0.10	0.13	0.06
Leaf width (cm)	3.0	2.7	2.5	1.5	1.2	1.3	1.2	4.5	4.4	3.1
SD	0.8	0.8	0.5	0.2	0.2	0.1	0.3	0.6	0.6	0.9
Leaf length (cm)	22.0	19.0	19.0	10.4	11.7	13.6	11.3	20.0	21.0	14.0
SD	7.1	5.0	2.7	1.1	1.0	1.4	0.5	1.4	3.9	2.8
Width/length	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.2
SD	0.02	0.01	0.02	0.02	0.01	0.01	0.03	0.02	0.02	0.02
Flower width (cm)	2.08	1.96	2.02					1.48	2.03	1.92
SD	0.30	0.09	0.29					0.20	0.33	0.11
Flower length (cm)	2.60	3.00	3.18					2.40	2.88	2.70
SD	0.14	0.31	0.25					0.10	0.25	0.23
Width/length	0.80	0.66	0.64					0.62	0.71	0.71
SD	0.15	0.07	0.08					0.09	0.13	0.04
Flower color	2	2	2+					2	2.5	2+
Distance between flower and closest leaf (cm)	0.54	0.66	0.58					1.14	0.70	0.94
SD	0.05	0.11	0.18					0.72	0.24	0.63

* 0 - white; 1 - light blue; 2 - blue; 3 - purple; 4 - dark purple

2.2.3b Hybridization of additional germplasm with var. ethiopica

In 1993 the following accessions were crossed with accession V-001 in two greenhouses: V-029, V-018, V-025, V-026, and V-020. Control pollination was carried out within each accession. The control crosses yielded few seeds, which leads us to believe that the seeds produced in the cross-pollinated flowers are indeed hybrid seeds. Pollination was completed by June 1993, and flower heads of each cross were collected. Data on the number of crosses and the number of seeds obtained are presented in Table 2.6. It should be mentioned that not all crosses yielded seeds.

Table 2.6. Statistics of hybrid seeds (F1) collected in the plasic-covered greenhouse from different pots (two plants per pot) during the season of 1992/3. Values represent number of capitula collected (N), mean and standard error of seeds per capitulum.

	Pot											Grand	Grand
Cross	1	2	3	4	5	6	7	8	9	10	N	Mean	sum
V-026 X V-001													
N	3	2	20	1	20	18	13				77		
Mean	4.7	36.5	12	14	18	60.9	1.7					23.7	
SE	2.9	1.5	4.8		3.6	12.8	1.4						
Sum	14	73	247	14	356	1096	2.2						1822
V-029o X V-001													
N	20	13	12	19	20						88		
Mean	14	14.9	29	21	16							18.6	
SE	3.2	2.52	2.8		4								
Sum	288	208	378	418	343								1635
V-029g X V-001													
N	32	22	15	8	30						107		
Mean	22	26.5	16	24	13							19.6	
SE	3.7	4.72	5.1	5.8	3								
Sum	693	582	245	191	388								2099
V-020 X V-001													
N	16	24	10	13	21	24	20	16	17	10	171		
Mean	19	50	7.5	12	27	8.42	6.8	6.19	1.5	1.1		16.3	
SE	8.7	10.1	5.1	3.2	8.4	2.1	2.6	2.45	1.2	0.7			
Sum	310	1201	75	153	577	202	136	99	26	11			2790
V018 X V-001													
N	13	18	19	20	17	27	27	36	23	27	227		
Mean	18	7.44	4.5	3.8	5.2	0.04	0.6	1.44	0.7	0.4		3.17	
SE	6.5	2.73	1.6	1.1	2.4	0.04	0.2	1.44	0.4	0.2			
Sum	240	134	85	75	88	1	16	52	17	12			720

This was more pronounced in the cross between V-018 and V-001, of which about only a mean of three seeds per flower head were produced in the crosses, whereas

16-24 seeds were obtained with crosses of other accessions with V-001. In this respect, variation was also noted between plants (Table 2.6).

F1 hybrids of from the 1993 season were grown both in the greenhouse and in the field in the winter of 1993/94 and the spring-summer of 1994, respectively, as described in Materials and Methods. We recorded time of flowering and characterized the plants phenologically. The F1 hybrids between the two ssp. *mutomoensis* (V-018) and *afromontana* (V-020) and ssp. *galamensis* var. ethiopica (V-001) flowered in the field two weeks earlier than the mother plants (Table 2.7).

Table 2.7. Flowering dates of capitula of different development stages of parents and hybrids of vernonia grown in the field in 1994. Seeds were sown in speedling trays on January 1 and transplanted in the field on February 2, 1994.

			Development stage		
Subspecies	Variety	Accession	I	II	III
galamensis	ethiopica	V-001	None	None	None
Nairobensis	-	V-026	3-7-94		
F1 hybrid		V-026 X V-001	3-7-94		
Mutomoensis	-	V-018	5-6-94	19-6-94	
F1 hybrid		V-018 X V-001	22-5-94	19-6-94	
Afromontana		V-020	5-6-94	19-6-94	
F1 hybrid		V-020 X V-001	22-5-94		
galamensis	petitiana	V-029g	12-4-94	8-5-94	5-6-94
F1 hybrid		V029g X V001	12-4-94	22-5-94	22-5-94
galamensis	petitiana	V-029o	12-4-94	22-5-94	19-6-94
F1 hybrid		V029o X V001	12-4-94	22-5-94	22-5-94
F2 hybrid		V029o X V001	22-5-94	22-5-94	19-6-94

Other F1 hybrids, those between ssp. *nairobensis* (V-026) or var. petitiana (V-029) with var. ethiopica, flowered at the same time as the mother plants. The earliest var. ssp. to flower was var. petiniana - 2.5 months after transplanting and the latest was ssp. *nairobensis* and its F1 hybrid with var. ethiopica - five months after

transplanting. The long time that it takes ssp. *nairobensis* and its F1 hybrid to flower is the reason that ssp. *nairobensis* was excluded from further hybridization work, which will be carried out in 1995. As in previous years, all accessions flowered much earlier when grown under short day conditions (in winter). In the winter plants are grown in the greenhouse to facilitate growth and to prevent frost damage and death. In the greenhouse flowering occurred two weeks after transplanting for var. galamensis (V-004) and var. petitiana (V-029) and up to eight weeks after transplanting for V-001 (Table 2.8). It should be noted that the F1 hybric between var. petitiana and ethiopica flowered at an intermediate date (three-four weeks after transplanting) as compared with their parents. Although the parents of ssp. *mutomoensis* (V-018) and ssp. *afromontana* (V-020) were not planted in this trial, their hybrids with var. ethiopica flowered after a similar period to that of var. ethiopica. Thus, with regard to flowering these hybrids display no advantage over var. ethiopica. In contrast to the situation in the greenhouse, parents of the hybrid were planted in the field along with their hybrids. In general, hybrid vigor was not observed, perhaps excluding ssp. *nairobensis*, in which the hybrid plants were bigge and had more secondary branches of increased length (Table 2.9). This is also reflected in the rate of growth as measured every two weeks during the growing season (see Fig. 2.1). The hybrid plants also differed in flower color and in involucr and peduncle length. The hybrid plants between ssp. *mutomoensis* (V-018) and var ethiopica were taller than both parents and intermediate in width. Otherwise, these hybrids were similar in characteristics to their mother plants (V-018), suggesting tha no hybridization took place.

As mentioned above, F1 hybrids of the 1993 season were grown in the greenhouse in the winter of 1993/94 together with plants of ssp. *galamensis* var. galamensis (V-004). Hybridization took place between var ethiopica (V-001) and both the F1 hybrids (back crosses) and accession V-004. The hybrid seeds were collected in summer, analyzed and stored. The seeds will be sown in the spring of 1995, and plants will be characterized during the following summer.

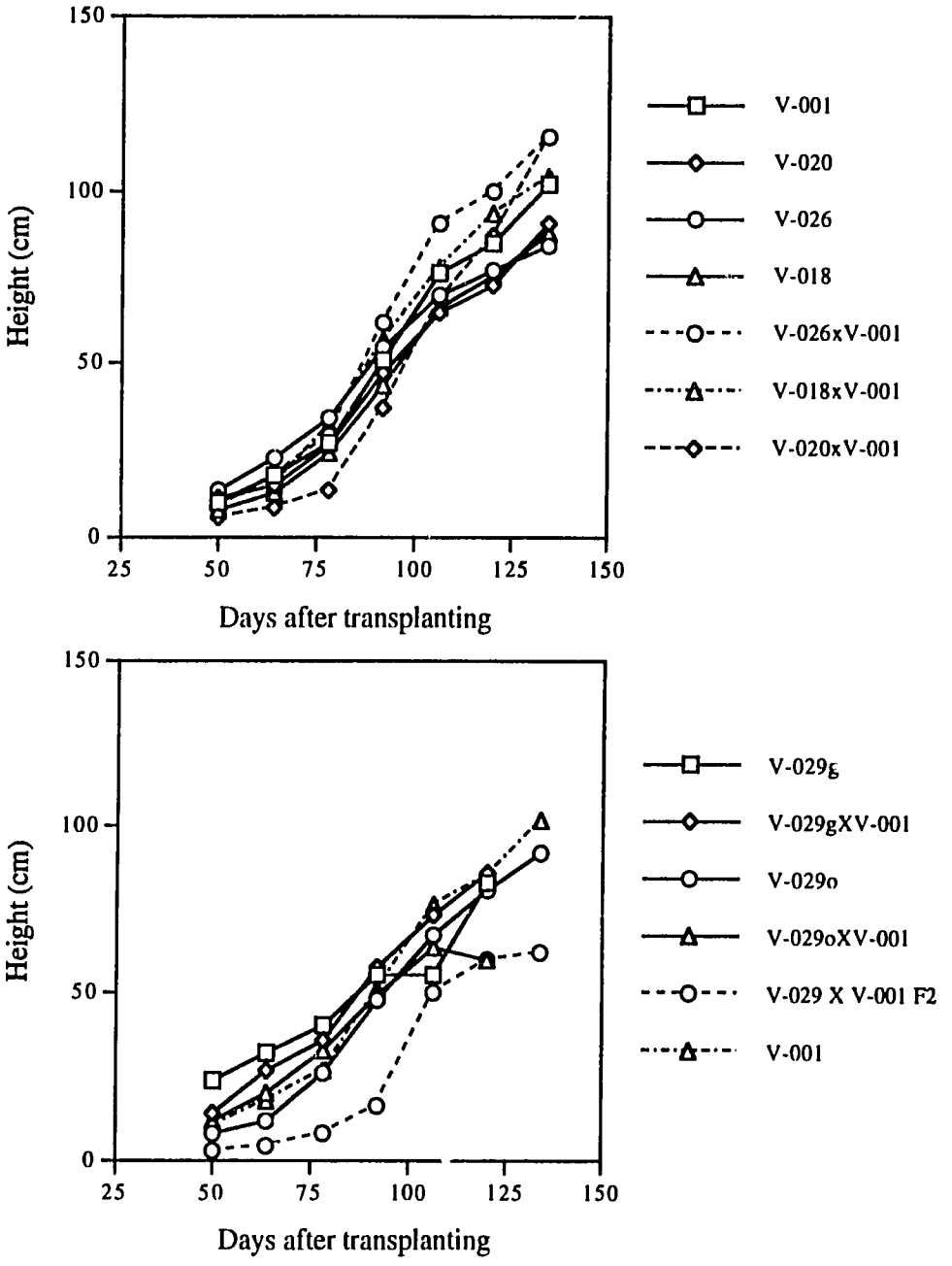


FIG 2.1 Growth of vernonia accessions and hybrids in the field during spring and summer of 1994.

Table 2.8. Flowering dates of capitula of different development stages of parents and hybrids of vernonia grown in the glass-covered greenhouse and the plastic-covered greenhouse in the winter of 1993/4. Seeds were sown in a petri dish on 5/10/94 and transplanted into pots on 11/10/94

Subspecies	Variety	Accession	Development stages I	II	III
Glass-covered greenhouse					
galamensis	ethiopica	V-001	18-25/11	23-29/11	2-11/12
galamensis	galamensis	V-004	25-31/10	30/10-5/11	7-12/11
galamensis	petitiana	V-029o	20-30/10	30/10-5/11	3-18/11
F1 hybrid		V029o X V001	28/10-7/11	31/10-11/11	4-20/11
F1 hybrid		V029g X V001	4-21/11	7-30/11	17/11-19/12
F1 hybrid		V-020 X V-001	15-25/11	17-30/11	15-17/12
F1 hybrid		V-018 X V-001	21-23/11	23-28/11	6-9/12
Plastic-covered greenhouse					
galamensis	ethiopica	V-001	22/11-5/12	28/11-8/12	5-26/12
galamensis	galamensis	V-004	20/10-7/11	30/10-23/11	5/11-2/12
galamensis	petitiana	V-029o	27/10-5/11	4-7/11	11-23/11
F1 hybrid		V029o X V001	13-14/11	18-20/11	26-28/11
F1 hybrid		V029g X V001	17-20/11	21-24/11	29/11-5/11
F1 hybrid		V-020 X V-001	30/11-3/12	3-6/12	5-13/12
F1 hybrid		V-018 X V-001	26/11-11/12	29/11-9/12	5-27/12

Table 2.9. Phenological characteristics of vernonia accessions and hybrids grown in the field in 1994. Values are average of 2-4 plants.

Phenological parameter	V001	V026	V026 X V001	V018	V018 X V001	V0290	V029g X V001	V029o X V001	V029X V001 F2	LSD
Plant height (cm)	105.0	108.0	110.0	108.7	127.0	96.2	69.0	75.3	72.3	25.5
Plant width (cm)	46.8	38.0	49.0	67.3	55.7	97.2	n.d.	67.0	37.0	27.1
Height of first flower (cm)	-	104.0	94.7	79.7	82.7	25.2	20	18.0	18.8	34.9
No. of 2ndary branches	-	14	37	33	38	20	10	6	13	15.1
Length of 2ndary branches (cm)	-	13.5	38.1	56.3	58.9	28.3	26.3	25.5	25.7	8.7
2nd branch diameter (cm)		5.1	5.5	5.9	5.7	7.9	6.0	8.2	6.9	1.8
Main stem diameter (cm)	4.7	1.8	2.4	2.5	2.3	2.1	2.5	2.0	1.4	7.2
Leaf length (cm)	15.9	21.3	21.2	16.6	17.3	11.3	13.3	11.0	11.8	1.6
Leaf width (cm)	1.1	3.1	3.2	2.0	2.0	1.0	0.9	1.1	1.1	0.2
Length/width	14.33	6.82	6.69	8.4	8.95	12.3	17.7	10.0	11.1	4.0
Flower color *	-	3	1-2	0-2	1-3	2	2-3	0-3	1	
Involucre length (cm)	-	14.8	13.2	16.5	17.4	15.7	15.4	13.5	15.0	1.1
peduncle (cm)	-	3.5	6.5	3.9	5.7	7.6	7.4	7.0	5.2	2.3

* 0 - white; 1 - light blue; 2 - blue; 3 - purple; 4 - dark purple

2.3. Vernonia germplasm - activity in Kenya

2.3.1. Germplasm collection

In collaboration with the National Gene Bank of Kenya, some germplasm collection missions were undertaken. The missions covered the lower parts of the Eastern Province, the western part of Coast Province, the Nairobi area, the Kiambu District, West of the Rift Valley (Western Kenya), and the Embu and Meru districts. The various routes were chosen in accordance with the information held at the Herbarium section of the National Museum of Kenya. The areas covered and the number of accessions collected are given in Table 2.10

Table 2.10. Germplasm collection in Kenya

Area	No. of acessions collected
Wundanyi	3
Machakos/Makeuni and Lower Kitui Districts	20
Nairobi Area/Kiambu District	8
West of Rift Valley/Western Kenya	18
Embu and Meru Districts	4
Total	53

The collecting team agreed on a standard strategy to be used during the collection missions. It was agreed that depending on the topography of the area, stops were to be made every 5 to 10 km on a chosen transect. At every stop, an area was thoroughly searched for the target plants. If the area through which the transect passed was hilly, stops were made strictly at a 5-km intervals, including hill tops, slopes and valley bottoms; but if the area was fairly uniform in topography, stops were made after every 10 km.

2.3.2 Germplasm characterization

The collected accessions are currently being characterized at two sites, namely:

i) Kibwezi (Dry Land Field Station of University of Nairobi). This site is representative of marginal lands.

ii) Muguga (Gene Bank Field) - The site is situated in an area of high agricultural potential areas.

The description list that is used was developed in accordance with the taxonomy of the plant. The list is appended to this report (Appendix).

Preliminary observations indicate that morphologically there are quite a lot of variations both within and between accessions. There is also variation between the two sites. At Kibwezi, for example, plants seem to be smaller in size and faster in maturing and have more flowers and higher fruit set than those at Muguga. Accessions seem to differ in maturity time and in flower and fruit production. Some accessions seem to be more prone to fruit shattering than others. This suggests that there is variation in the accessions which will be useful in breeding programs to develop cultivars. It has been noted, for example, that some accessions have comparatively large fruits with good seed retention.

2.3.3 Future Plans

—Collect and analyze data on characterization

—Verify some of the data that are not clear

—Perform agronomic trials on the effects of topping and plant density on the expression of various important traits

—Separate the accessions into their taxonomic groups - varieties and subspecies

—Organize another collecting expedition

APPENDIX

Descriptor list for *Vernonia galamensis*

1. Days to emergence - number of days from sowing to 50% emergence.

2. Seedling vigor - visual measurement recorded three weeks after emergence of the seedling
   1 - Very strong
   2 - Strong
   3 - Average
   4 - Weak
   5 - Very weak

3. Days to 50% flowering - number of days from planting to time that 50% of plants have flowers

4. Synchrony of flowering - whether the main stem and branches flower at the same time
   1 - Synchronous
   2 - Non-synchronous

5. Number of flower heads per plant - mean of five randomly selected plants

6. Number of branches per plant - mean of five randomly selected plants

7. Branching habit
   1 - Upright
   2 - Spreading
   3 - Drooping

8. Plant height (cm) - mean of five randomly selected plants taken after maturity of the terminal pod from ground level to the tip

9. Leaf length (L) - measured in mm as the mean of five randomly selected leaves per plant from five plants taken at flowering

10. Leaf width in mm - taken together with leaf length

11. Leaf length/width (L/W) ratio

12. Leaf shape
    1 - Circular - very narrow, stiff and pointed
    2 - Linear - long and narrow with parallel edges
    3 - Oblonceaolate - reverse of conceolete i.e. broadest near the top.

13. Flower color - the predominant flower color
    1 - White
    2 - Purple
    3 - Blue
    4 - Yellow with purple
    5 - Specify

14. Seed color - main seed color at maturity
    1 - Black
    2 - Brown
    3 - Grey
    4 - Other (specify))

15. Stem pubscence - stem hairiness
    0 - Glabrous
    3 - Sparse
    5 - Intermediate
    7 - Abundant

16. Leaf pubscence - leaf hairiness
    0 - Glabrous
    3 - Sparse
    5 - Intermediate
    7 - Abundant

17. Stem thickness (cm) - measured 30 cm from the ground at maturity as means of five randomly selected plants

18. Stem color - visual observation of the stem taken at flowering

19. One-thousand-seed weight

20. Number of seeds per pod - average of five randomly selected pods from five plants

21. Inflorescence growth habit
    1 - Lax
    2 - Very lax
    3 - Other (specify)

22. Phylaris growth habit
    1 - Drooping
    2 - Spreading
    3 - Acute

23. Flower head shape
    1 - U-shaped
    2 - Other (specify)

24. Oil content - percentage oil content in 20-g seed weight

25. Disease incidence - recorded as a percentage of the number of cases reported
    1 - Below 0%
    3 - Up to 25%
    5 - Up to 50%
    7 - Up to 75%
    9 - Up to 75%

26. Pest incidence - as for diseases

27. Leaf senescence - amount of leaves remaining when 75% of the pods are mature
    3 - Few leaves remaining
    5 - Intermediate
    7 - Most leaves remaining

28. Days to 90% maturity - days from sowing to 90% maturity.

29. Lodging - susceptibility in lodging scored at pod maturity.
    0 - No plant lodged
    1 - Very few lodged
    5 - Almost 50% lodged
    9 - Nearly all plants

30. Seasonality - life span
    1 - Annual
    2 - Biennial
    3 - Perennial

31. First harvesting - days from sowing to first harvesting

32. Germination percentage

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C) Scientific Impact of Collaboration

All four collaborating scientists have participated in the project since its initiation, each one in his/her field. All the activities are described in Section I part B. There was also interaction between the scientists, which is described below in Section II part D. All four collaborating scientists are in a continuous contact for updating research activity and progress.

D) Description of Project Impact

So far, the results obtained in the project are being used only within the project itself, in the process of progressing with the work. It is hoped that by the end of the project some industrial applications may be initiated.

E) Strengthening of Developing Country Institutions

The main investment in Israel was in the purchase of a gas chromatograph (part of the money came from the AID/CDR project). In Kenya, so far, small appliances have been purchased for the chemistry laboratory: a magnetic stirrer, a semi-analytical electronic balance, a heating mantle, and a jack. These appliances are needed for oil extraction and as such will strengthen the research working capacity of the chemistry laboratory. In September, Ms. M. Wawira from Kenya came for a training period of one year in the chemistry laboratory of Dr. S. Grinberg. Ms. Wawira is a M.Sc. student at the University of Nairobi; her work in Israel constitutes part of her thesis requirements which will be carried out under the supervision of Prof. P. Gitu and Dr. S. Grinberg. Since her arrival Ms. Wawira has strengthened her skills significantly. She is working with new procedures of chromatography such as HPLC and new analytical procedures, such as infra red and is learning new determinations, such as free fatty acid level. There is no doubt that upon her return to Kenya she will contribute to her department by teaching her colleagues the new techniques she has aquired in Israel.

F) Future Work

Basically, with regard to the working plans presented in the proposal, the program both in Kenya and Israel is on schedule. We initiated the work in 1992 (before final approval in mid 1993), since there was a delay in the process of approving this program. A revision in the strategy of hybridizing subspecies and varieties was made after the research proposal had been and submitted: We originally noticed that two plants of ssp. *mutomoensis* (accession V-025) produced no seeds whatsoever (being self-sterile). Since we were able to vegetatively propagate those

plants by cuttings, we thought that it will be easy to prepare self-sterile plants of other accessions. We subsequently realized that most plants, with the exception of var. ethiopica (V-001), are quite self-sterile in the greenhouse. This characteristic, which is inferior agronomy-wise enables us to perform hybridizations without selecting self-sterile plants, at least with some of the accessions that we use for crossing.

SECTION II

A) Managerial Issues

The main managerial concern that arose in the first year regarded the late start of the project in Kenya, due to the delay in the transfer of money. We feel that an extension of the program should thus be granted. These problems and a request for an extension were put in a letter to Mr. Boaz Ayalon, at the American Embassy in Tel Aviv. In response, we received a letter granting us the requested extension (until August 1998). We thank AID/CDR for this and are convinced of its benefitial to the project.

B) Budget

So far there are no major changes in budget

C) Special Concerns

Currently not relevant

D) Collaboration, Travel, Training and Publications

The two Israeli scientists, Dr. D. Mills and Dr. S. Grinberg, are in close collaboration. This collaboration is expressed in: i) managerial issues such as allocation of budget, supervision of the trainee from Kenya and contact with our collaborators from Kenya, and ii) scientific aspects, mainly in growing seeds for oil production and in the preparation of the crude oil. The close collaboration described is also true between the two Kenyan partners.

There were two meetings between the Kenyan and Israeli partners: the visit of Dr. D. Mills to Kenya and the visit of Prof. P. Gitu to Israel.

Dr. D. Mills spent 10 days in Kenya at the begining of July 1994. He joined a short germplasm collection in the dry Kibwezi area with Prof J. Chweya, visited three of the germplasm trials at Kibwezi and Muguga, and helped in the classification and characterization of the plants. He also visited the laboratory of Prof. P. Gitu and discussed the chemistry part of the project. The trip to Kenya was coupled with a trip to Ethiopia to examine a promising population of vernonia in the southern part of the country (see details of the entire trip in section I part B)

Prof. P. Gitu visited the laboratory of Dr. S. Grinberg in Beer-Sheva for five weeks from mid-August to mid-September 1994. He was accompanied by his student Ms. M. Wawira who will spend 12 months in the chemistry laboratory working on amination of the vernonia oil. The Kenyan scientists discussed the program with Dr. S. Grinberg and initiated some experiments.