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CHEMICAL STUDIES ON VERNONIA OIL AND GENETIC IMPROVEMENT OF *VERNONIA GALAMENSIS*

Final Report
August 1993 - December 1998

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1 Executive Summary

In the past few years there has been a growing consensus among the industrialized nations of the world regarding the need to find raw materials from sustainable and renewable resources rather than from finite petroleum resources, which are constantly being depleted as sources of energy and chemical feedstocks The objectives of our AID-CDR project were to study the chemistry of the very special epoxy oil of the vernonia plant and to synthesize novel products with potential industrial applications, and concomitantly to broaden our collection of germplasm of the plant by collecting from the wild and performing intraspecific hybridization, as well as to carry out commercial trials in Kenya *V galamensis*, which occurs naturally in Kenya, could offer a significant opportunity for sustained development, especially in marginal land which awaits further development

In collaboration with the National Gene Bank of Kenya, germplasm was collected by Prof Chweya in a mission to different Provinces A total of 53 accessions were collected representing four sub-species of *Vernonia galamensis* ssp *nairobensis*, ssp *gibbosa*, ssp *mutomoensis*, and ssp *galamensis* (two varieties *ethiopica* and *petitiana*)

The collected material was subject to varietal trials at two sites in Kenya, Kibwezi Dry Land Field Station and Kabete Field Station of the University of Nairobi, representing respectively marginal and high potential land Considerable differences in phenological characteristics and yield were found among the 43 accessions of the four subspecies At Kibwezi Station the highest-yielding accessions were, on average, those of ssp *mutomoensis* (2 tons seed per hectare) However, at Kabete Station yields were much higher ssp *gibbosa* yielded 3 t/ha

Further field testing aimed at commercialization was conducted in the same stations in Kenya under dryland farming conditions The somewhat disappointing yields obtained at the two sites—up to 0 48 t/ha at Kabete and 0 28 t/ha at Kibwezi—appear to have been due to a dry spell during the period of growth However, potential yielding of vernonia should be re-examined The possibility of irrigating the crop in order to secure high and reliable yields should be explored

Since none of the vernonia germplasm collected is appropriate for cultivation in Israel or in other areas situated far from the tropical zone, we set out to develop a line with suitable characteristics through introgression of the desirable characteristics of ssp *galamensis*, var *ethiopica*—big capitulum (flowerhead), big seed, high seed retention (low shattering), and high self compatibility—into other subspecies and varieties possessing different desirable characteristics—numerous capitula and neutral day flowering (mainly ssp

galamensis varieties *galamensis* and *petitiana)* A wide variety of crosses were carried out at the start of the project, and selfed offsprings were selected for five generations as the project unfolded Most promising were the hybrids between var *ethiopica* and var *galamensis* We observed that the characteristics of the hybrid lines were generally intermediate between their two parents Flowering, consistently recorded about three months from sowing, occurred only 10-14 days later than in var *galamensis* This is one of the important characteristics of the hybrids Number of flowerheads, which was low and quite variable, is the major disadvantage of the hybrid lines developed through this program, though it is similar to that of the parent lines

The chemistry of vernonia oil was studied extensively and in depth New chemical derivatives and new polymers based on vernonia oil as a renewable resource were prepared by exploiting the chemical multi-functionality of the oil Reactions were performed on the three functional groups Brominated compounds were obtained by performing addition and allylic bromination on the double bonds of the methyl vernolate and the oil itself Aminolysis of vernonia oil was performed by reacting the oil with aliphatic diamines to obtain the corresponding mono- and divernolamide The fatty bisamides have two epoxy groups in the molecule and represent new reactive difunctional epoxy monomers By reacting vernonia oil with aromatic diamines, a series of new rubber-like polymers were obtained The gel fraction content of the cured polymers ranged between 45% and 70%, and as expected they were more thermostable than the original vernonia oil

The polymers were then used to modify commercial epoxy resin (Epicote 828) in order to obtain interpenetrating polymer networks (IPNs) The formulations were prepared by adding prepolymers of vernonia oil with *m*-phenylene diamine (or with epoxol with *m*-phenylene diamine, for comparison) to a mixture of the epoxy resin in the presence of an accelerator The formulations were then mixed at room temperature to produce homogeneous transparent mixtures The transparency of the mixtures obtained is evidence of the compatibility between the epoxy resin and the corresponding prepolymer The IPN containing 50% vernonia oil polyamine, for example, is a reinforced elastomer with an elongation that is three times greater than that of the polyamine of vernonia oil

Acrylation and methacrylation of vernonia oil gave new reactive a-olefinic monomers These multifunctional acrylates (MFAs) were photochemically cross-linked, in the presence of benzophenone, to produce transparent colorless films with good mechanical properties The polymethacrylate of vernonia oil was used as elastomer to modify epoxy resins to obtain IPNs The polymers derived from vernonia oil/aromatic diamines, in terms of their thermostability and elastomeric properties as well as the simplicity of synthesis,

represent a promising pathway to the synthesis of new IPNs The polymers derived from acrylates (methacrylates) of vernonia oil, which are prepared by an energy-efficient and pollution-free method, represent another such promising pathway

This project has enabled a group of scientists in Kenya and Israel to acquire a wide-ranging fund of knowledge about a special new crop, *Vernonia galamensis*, and the unique oil that accumulates in its seeds The members of the group are now very well acquainted with the natural habitats of the plant, its biology, agronomic practices needed for growing it, the methodology for hybridizing between different germplasms within the species, how to extract the oil, analyze it, and prepare novel products from it using the different functional groups, and how to characterize the new products (especially polymers) prepared from the oil and analyze their properties Know-how generated by the scientists of the project was disseminated at meeting and through the medium of scientific papers Four Kenyan graduate students completed their MSc studies in the framework of this project, one was trained in Israel Work on the project is being continued despite the formal termination of funding while retaining the original main objective of developing *Vernonia galamensis* as a new industrial crop for African populations

2 Research Objectives

Vernonia galamensis is a native plant which grows in Africa Its seeds contain about 40% of a unique oil (epoxy triglyceride) which has great potential in the chemical industry, both as an alternative to existing products (epoxidized soybean oil, or ESBO, and epoxidized linseed oil, or ELSO) and as a basis for preparing novel products ESBO and ELSO are currently prepared by epoxidation of the crude vegetable oil, a process which doubles or triples the original cost For vernonia oil (VO) to compete successfully with them economically, it is necessary to develop improved agrotechniques and germplasm and, most importantly, novel uses and products that would be difficult to derive from other oils, which are characterized by random epoxidization

In Kenya, because of rapid population growth, there is strong pressure on fertile farmland for subsistence and cash crop agriculture However, the fertile parts account for only about 20% of the country Kenya's Fifth Development Plan focuses, therefore, on developing appropriate technology for exploiting the arid and semiarid regions of Kenya, which occupy about 75% of the total area These regions are currently inhabited by six million people and are becoming more and more densely populated Prior attempts to establish alternative crops in these arid and semiarid regions failed because (a) added value and demand for the final products were insufficient to ensure profitability, (b) superior production required additional inputs such as irrigation, and (c) introduced plants were not able to adapt to the local habitat *V galamensis*, which occurs naturally in Kenya, including in the semiarid areas, could offer a significant opportunity for sustained development

Our overall objective was to develop *V galamensis* as a new industrial crop for semiarid zones In accordance with the rationale set out above, five objectives were identified, as follows

1 to broaden our germplasm collection,
2 to perform subspecies and varietal trials in a semiarid zone in Kenya,
3 to carry out a commercial trial in Kenya,
4 to carry out intraspecific hybridizations to introgress traits from different subspecies and varieties into elite plants,
5 to study the chemistry of the very special epoxy oil of the vernonia plant and synthesize novel products with potential in industry

3 Methods and Results

The following chapters summarize the results and accomplishments of this program They are organized according to the objectives stated above

To our great and lasting sorrow, Prof James Chweya passed away during the last stage of the project Since he was not able to sum up the work for the final report in line with the specific questions and comments sent to him, Dr David Mills has tried to do so We are aware that the result is unavoidably inferior to the summation as it would have been drawn up by the late Prof Chweya

3 1 Expanding the germplasm collection

A trip made by Dr D Mills to Southern Ethiopia led to the identification of interesting *Vernonia* germplasm in the wild (Mills et al 1994) The significance of the populations found in Ethiopia lies in their relatively short growing season, the timing of flowering (July to August), and the relatively low water requirement for completion of the life cycle (Seeds were not ripe at the time of the visit) The fact that these newly discovered specimens flower under long-day conditions makes them very attractive as candidates for cultivation in areas further north and south of the equator Indeed, of all the vernonia accessions tested so far at sites far removed from the equator, only a few flower and set seed in summer (Thompson et al 1994), and these few are inferior to ssp *galamensis* var *ethiopica* The latter sets seed in winter, and since it is susceptible to cold, it cannot be cultivated in regions such as Israel and the Southern USA As far as we know, plants of the populations described above have never been tested outside of Ethiopia Dr Mills also participated in a brief germplasm collection expedition in the dry Kibwezi area with his Kenyan partners

In collaboration with the National Gene Bank of Kenya, germplasm was collected by Prof Chweya (Mills et al 1995) in a mission to southern parts of Eastern Province, Western part of Coast Province, the Nairobi area, Kiambu District, West of the Rift Valley (Western Kenya), and Embu and Meru Districts The various routes were chosen in accordance with information available in the Herbarium section of the National Museum of Kenya A total of 53 accessions were collected representing four sub-species of *Vernonia galamensis* ssp *nairobensis,* ssp *gibbosa,* ssp *mutomoensis,* and ssp *galamensis* (two varieties *ethiopica* and *petitiana*) (var *ethiopica* does not grow indigenously in Kenya and is probably part of the collection of the National Gene Bank of Kenya - D Mills)

3 2 Variety trials

The variety trials were performed at two sites Kibwezi Dry Land Field Station and Kabete Field Station of the University of Nairobi The former represents marginal lands while the latter represents high potential areas The trials were designed in order to contribute towards meeting two of the project's specific

objectives searching for the most promising germplasm, and checking for adaptability of germplasm in the semi-arid zones of Kenya

Considerable differences in phenological characteristics and yield were found among the 43 accessions of the four subspecies (Mills et al 1996) (Table 1) At Kibwezi Station, accessions of ssp *mutomoensis* gave on average the highest yields (2 tons seed per hectare) and those of var *ethiopia* the lowest (0 8 ton seed per hectare) (Table 2) At Kabete Station, yields were much higher ssp *mutomoensis* yielded on average 0 9 t/ha and ssp *gibbosa* 3 t/ha Topping (removing the apical part of the main stem) was found to have a significant effect on some phenological characteristics, but did not cause any change in seed yield No significant effect of plant density on yield was found (Mills et al 1995)

In a second trial (Mills et 1997), several accessions representing the different subspecies were tested in small 3x3 m plots at the two stations mentioned above Seed yields were similar at the two locations but were much smaller than in the previous season 0 06-0 2 t/ha The low yields were the result of a dry spell

When extrapolated from the small plots (4 m^2), the high yields (0 7-4 tons seeds per hectare) reported by the Kenyans (first variety trial, Mills et al 1995) are very encouraging and are higher than the few figures reported in the literature so far (Perdue et al 1989 a)

Table 1 Phenological characterization of 43 vernonia accessions grown at Kibwezi

Accession	Collection site	Days to maturity	Height at flowering (cm)	Height at maturity (cm)	Stem diameter (cm)	Leaf length (cm)	Leaf width (cm)	L/W Ratio
GBK 013234	Kisii	126 0	113 5	106 5	1 6	16 5	3 0	5 5
GBK 034514	Kisii	132 0	143 7	143 7	1 7	22 3	3 0	7 4
GBK 034523	Trans Mara	130 0	140 7	183 0	1 4	23 0	4 3	5 3
GBK 034513	Kisii	138 7	142 7	149 7	1 5	21 3	3 3	6 5
GBK 034522	Kisii	136 3	134 3	171 3	1 7	22 7	3 3	6 9
GBK 034498	Kitui	116 7	66 7	161 7	1 5	15 3	2 0	7 7
GBK 034506	Kiambu	137 0	156 7	185 7	1 9	22 3	3 0	7 4
GBK 034496	Kitui	120 3	77 7	121 7	1 5	15 3	3 7	4 8
GBK 006202	Baringo	135 7	101 3	154 3	1 2	17 0	2 0	8 5
GBK 034491	Makueni	113 7	67 7	143 0	1 7	16 0	2 0	8 0
GBK 034521	Trans Nzoia	150 0	152 3	159 0	1 6	22 3	3 3	6 8
GBK 034586	Meru	117 0	91 0	129 0	1 6	16 0	4 5	3 6
GBK 006210	Kiambu	138 0	159 3	199 3	1 8	23 3	3 3	7 1
GBK 034520	Trans Nzoia	145 3	148 3	163 0	1 7	22 7	3 3	6 8
GBK 034518	Kisii	131 0	121 0	148 3	1 5	20 0	3 0	6 7
GBK 034584	Nyamira	121 3	84 0	141 0	1 6	17 3	3 7	4 8
GBK 034492	Makueni	120 0	84 0	139 0	1 5	17 0	3 0	5 9
GBK 034502	Kiambu	126 3	116 0	162 3	1 6	18 0	3 0	7 3
GBK 006213	Kiambu	133 0	123 3	182 7	1 5	20 7	3 0	6 9
GBK 034509	Kisii	137 7	153 0	172 0	1 6	21 7	4 0	5 7
GBK 034493	Kitui	115 3	67 3	143 3	1 7	16 7	2 3	7 4
GBK 034515	Kisii	127 0	133 3	195 0	1 7	24 3	4 0	6 1
GBK 034505	Kiambu	130 3	121 7	159 3	1 8	19 3	3 0	6 4
GBK 034507	Kiambu	139 3	143 0	196 7	1 6	22 0	3 7	6 1
GBK 034516	Kisii	137 7	128 3	179 0	1 7	21 3	3 7	5 9
GBK 034490	Makueni	114 7	80 0	152 0	1 7	15 7	2 7	6 6
GBK 034517	Kisii	124 3	118 3	161 0	1 6	20 0	2 7	7 7
GBK 034512	Narok	132 3	140 0	144 3	1 8	20 7	3 0	6 9
GBK 034511	Kisii	138 7	129 3	160 3	1 9	21 7	3 3	6 6
GBK 034479	Makueni	113 3	65 0	112 7	0 8	15 7	1 7	10 3
GBK 028722	Meru	117 0	84 3	126 0	1 6	16 7	3 7	4 6
GBK 034480	Makueni	114 5	80 5	109 0	1 6	17 0	5 0	3 4
GBK 028890	Nairobi	125 7	117 0	174 7	1 5	18 3	3 3	5 7
GBK 034501	Kiambu	125 0	100 7	139 0	1 7	18 0	4 3	4 2
GBK 006169	Machakos	110 7	69 7	124 3	0 9	16 0	1 3	14 7
GBK 034503	Kiambu	130 3	137 0	153 0	1 7	21 0	3 3	6 3
GBK 034587	Meru	122 0	88 3	139 0	1 6	17 0	3 3	5 3
GBK 006178	Taiat Taveta	118 0	98 3	122 3	1 7	20 0	3 0	6 7
GBK 034499	Kitui	113 7	74 3	146 7	1 7	16 3	2 0	8 2
GBK 034588	Meru	120 7	104 0	152 3	1 4	17 7	3 7	5 0
GBK 034510	Kisii	115 3	98 3	164 3	1 7	19 0	3 0	6 8
GBK 034504	Kiambu	142 3	84 3	151 7	1 4	17 3	3 0	8 3
GBK 034524	Bomet	129 3	120 7	149 7	1 9	20 7	3 7	5 7
Significance		**	***	**	***	***	***	***

** = 0 01>P>0 001, *** = P<0 001 L/W Ratio = Leaf length/Leaf width ratio

Table 2 Yield and yield components of 43 vernonia accessions grown at Kibwezi

Accession	Collection site	Days to 50% flowering	Branches (no per plant)	Flowers (no per plant)	Harvest period (days)	Seed yield (kg/ha)	1000 Seed wt (g)
GBK 013234	Kisii	102 0	28 0	28 5	57 0	397 50	4 50
GBK 034514	Kisii	94 3	18 3	29 3	52 3	708 33	5 33
GBK 034523	Trans Mara	99 3	15 0	34 3	54 3	506 25	5 50
GBK 034513	Kisii	101 7	13 7	34 7	47 0	691 67	4 67
GBK 034522	Kisii	96 7	17 0	30 3	50 7	516 67	5 33
GBK 034498	Kitui	65 7	13 7	51 3	74 0	1833 33	8 67
GBK 034506	Kiambu	101 3	21 7	33 0	46 0	441 67	5 67
GBK 034496	Kitui	74 0	11 3	48 0	61 3	1983 33	6 67
GBK 006202	Baringo	85 3	15 3	29 0	49 3	829 17	4 67
GBK 034491	Makueni	65 0	13 0	46 3	70 0	2283 33	6 67
GBK 034521	Trans Nzoia	114 0	12 7	13 3	28 0	137 50	7 33
GBK 034586	Meru	82 5	13 0	33 0	71 0	2056 25	9 00
GBK 006210	Kiambu	104 7	14 3	33 3	46 7	637 50	4 67
GBK 034520	Trans Nzoia	101 3	22 0	33 0	41 0	137 50	5 67
GBK 034518	Kisii	99 0	19 0	32 7	49 7	754 17	7 33
GBK 034584	Nyamira	72 0	13 0	40 7	63 3	1491 67	7 00
GBK 034492	Makueni	71 0	14 0	47 7	69 3	1404 17	7 67
GBK 034502	Kiambu	64 0	12 3	17 7	57 7	541 67	7 67
GBK 006213	Kiambu	102 7	14 7	27 3	52 3	1066 67	4 33
GBK 034509	Kisii	96 7	12 0	15 7	45 0	345 83	5 67
GBK 034493	Kitui	62 0	12 0	77 3	69 7	2166 67	6 67
GBK 034515	Kisii	99 3	19 0	43 7	59 3	550 00	5 33
GBK 034505	Kiambu	96 0	17 7	39 7	53 7	687 50	5 33
GBK 034507	Kiambu	98 0	14 7	27 7	40 7	425 00	7 33
GBK 034516	Kisii	94 7	19 0	29 7	44 3	675 00	6 00
GBK 034490	Makueni	66 0	13 7	50 3	67 3	2554 17	7 33
GBK 034517	Kisii	95 3	15 7	53 0	65 7	1329 17	7 00
GBK 034512	Narok	101 7	21 3	25 7	52 3	341 67	6 00
GBK 034511	Kisii	102 0	18 3	28 3	50 0	625 00	6 67
GBK 034479	Makueni	54 3	13 7	196 0	62 3	1016 67	4 33
GBK 028722	Meru	74 7	17 0	39 0	67 7	2166 67	4 67
GBK 034480	Makueni	76 0	14 5	45 0	75 5	1793 75	8 00
GBK 028890	Nairobi	94 7	14 0	48 0	63 0	1312 50	6 00
GBK 034501	Kiambu	82 0	16 7	51 7	65 0	1720 83	7 33
GBK 006169	Machakos	56 0	16 0	103 0	72 0	1704 17	4 67
GBK 034503	Kiambu	91 3	14 0	27 0	52 3	1162 50	8 67
GBK 034587	Meru	89 3	16 3	39 0	66 0	941 67	8 00
GBK 006178	Taiat Taveta	97 0	17 7	41 3	75 3	945 83	9 33
GBK 034499	Kitui	59 7	12 3	48 7	72 7	2816 67	7 67
GBK 034588	Meru	86 7	21 7	30 0	62 7	1262 50	8 00
GBK 034510	Kisii	80 3	14 3	43 7	73 0	937 50	7 67
GBK 034504	Kiambu	80 0	15 3	28 3	42 7	645 83	5 00
GBK 034524	Bomet	94 7	14 7	29 7	50 3	795 83	8 00
Significance		***	ns	***	***	***	**

ns = Not significant, ** = 0 01>P>0 001, *** = P<0 001

3 3 Commercial trials

In Kenya, further field testing—aimed at commercialization—was conducted (Mills et al 1998) The trials were conducted under dryland farming conditions Low yields were obtained at the two sites, up to 0 48 t/ha at Kabete and 0 28 t/ha at Kibwezi, due to a dry spell during the period of growth and to interference by wild animals (Table 3) Var *ethiopica* was more susceptible to the drought than the other subspecies tested At both locations, subspecies *mutomoensis* yielded more than the other germplasm

Table 3 Days to flowering, harvesting period, and seed and oil yields of *Vernonia galamensis* growing at Kabete and Kibwezi

Location	Subspecies/var	Days to 50% flowering	Harvesting period (days)	Seed yield (kg/ha)	Oil yield (kg/ha)
Kabete	Mutomoensis	103	118	475	147 1
	Gibbosa	116	118	378	127 4
	Ethiopica	129	104	351	108 6
Kibwezi	Mutomoensis	-	-	281 7	88 2
	Gibbosa	-	-	213 3	71 9
	Ethiopica	-	-	241 0	75 4
	Nairobensi	-	-	193 4	62 3

In another trial as well, differences in the ability of plants to tolerate or withstand dry spells were evident Var *ethiopica* was particularly affected by the drought, i e more so than the two subspecies *mutomoensis* and *gibbosa* (Table 4) Water stress was so serious in *ethiopica* that by the time the plants had attained 50% flowering, almost half the plants had dried up The surviving plants had fewer primary branches and hence fewer flowerheads The two subspecies tolerated water stress very well, with the crop remaining green throughout the growing period The plants were generally bushy (extensively branched) and formed many flowerheads Yields were 0 2-0 25 t/ha at Kibwezi

At Kabete plants of var *ethiopica* were subjected to clipping or not Clipped plants yielded (0 3 t/ha) more than non-clipped plants (0 2 t/ha), although the latter had more primary branches Many of the branches on the nonclipped plants were, however, small, and produced very few flowers as compared with branches on the clipped plants The latter showed extensive vegetative growth and also produced many flowers

Table 4 Vegetative and reproductive parameters of *Vernonia galamensis* grown at Kibwezi

Subspecies	No of primary branches	Days to first flowering	Days to 50% flowering	Days to maturity	Yield (kg/ha)
Gibbosa	12	72	81	105	196
Mutomoensis	14	72	89	105	253
Ethiopica	19	76	96	112	20

3 4 Intraspecific hybridization in vernonia

The main objective of the hybridization trials was to introgress desirable characteristics of ssp *galamensis*, var *ethiopica*—big capitulum (flowerhead), big seed, high seed retention (low shattering), and high self compatibility—into other subspecies and varieties possessing other desirable characteristics—numerous capitula and neutral day flowering (mainly ssp *galamensis* varieties *galamensis* and *petitiana)*

Below we describe the process of crossing and the general characteristics of the offspring and selected plants (inbred lines) in each of the five generations, dwelling especially on a specific cross that seemed more promising than the others at the first and also at a later stage

3 4 1 Crossing

Hybridization (crossing) was carried out simply by growing parent plants in pots in the greenhouse Each parent was planted in 3-8 pots lined up in a row, with parent lines to be crossed in parallel rows Branches bearing capitula were bent across the interspace between rows and rubbed against each other to exchange pollen Female capitula were then bagged and seeds collected after maturation To assess self-compatibility (selfing), controls were either bagged capitula or capitula that had been rubbed against others of the same plant

In the first year of the program we conducted some preliminary hybridization trials (Mills and Grinberg 1994b), these were described in our first annual report (Mills et al 1995) In a subsequent extended trial, we carried out 887 crosses (Mills et al , 1996), most performed between ssp *galamensis* var *ethiopica* (V-001) and two other varieties, *petitiana* (V-029) and *galamensis* (V-004), but some also with three subspecies, *nairobensis* (V026), *afromontana* (V-020) and *mutomoensis* (V-018) In most cases ssp *galamensis*, var *ethiopica* served as the male (donor of pollen) and in some cases, as with crosses with ssp *galamensis*, var *galamensis*, also as the female Self-compatibility was low in most germplasm used as female, that is, few seeds developed when capitula were selfed or just covered Also, backcrossing was carried out by crossing F1 hybrids of the cross var *petitiana* x var *ethiopica* (V029 x V-001) with the male parent V-001 Descendants of this backcross were not promising (Mills et al , 1996) and will not be described here

The grand average showed that crosses produced 19 seeds per head and selfing only 8-10 seeds per head (Mills et al 1995) This finding alone proved that crossing produces more seeds than the natural amount obtained by selfing The seeds collected from crossed flowerheads, however, could either be a product of the cross or the product of selfing, and therefore a thorough examination was carried out to screen the F1 line and descendants During the project, selfed offspring of five generations (inbred lines) were grown in the field and the greenhouse using basic and common practices in an effort to ensure proper growth of the plants With the years we have narrowed down the number of lines selected to one (1999) This superior line is a cross between

ssp *galamensis* var *galamensis* (V-004) and ssp *galamensis*, var *ethiopica* (V-001) In this final report we track down the path of screening and selection of the lines as carried out in the field and the greenhouse

The crosses of var *galamensis* (V-004) with var *ethiopica* (V-001) were performed in the glass-covered greenhouse in four different rows Apparently, there were differences between them In terms of differences in the number of seeds between the cross and the covered and control pollination, the crosses of plants within rows F and H were more successful (Mills et al 1995) An examination of the data for seed counts per plant (pot) revealed large differences between the crosses (Table 5) In some, selfing was high (for example, pot 3, row B, and pot 1, row D), casting doubt on the efficiency of these

Table 5 Mean number of seeds per capitulum in cross pollinated, control (selfed) and covered flower of crosses of ssp *galamensis* var *galamensis* (accession V-004) with var *ethiopica* (accession V-001)

Data were collected in different pots of different rows in a glass-covered greenhouse in the period November 1993-February 1994

Row	Pot	Crossed		Selfed			
				Covered		Rubbed	
		N	seed/head	N	seed/head	N	seed/head
B	1	14	9 4±3 1	5	5 4±4 2		
	2	4	11 0±3 1	2	2 5±2 5		
	3	15	6 3±2 4	4	11 8±10 4	1	0
	4	15	4 8±1 3	5	1 4±0 8		
D	1	15	26 2±4 0	6	33 7±8 0		
	2	12	7 1±2 1	2	0		
	3	14	6 7±2 1	4	1 0±1 0		
	4	11	10 8±4 4	4	0 8±0 8		
F	1	15	11 9±3 5	10	4 9±4 8	5	9 8±5 7
	2	9	9 6±4 7	4	0 8±0 5		
	3	7	11 7±5 2	3	0 3±0 3		
	4	23	11 7±3 3	5	4 6±4 1	1	0
	5	13	8 7±3 3	2	0		
	6	14	8 1±4 6	2	0		
	7	15	18 2±4 8	6	5 8±5 8	1	0
	8	9	9 2±5 4	4	0		
H	1	28	9 7±2 3	12	0	2	2 5±1 5
	2	6	19 5±7 9	2	0	1	0
	3	7	14 2±3 3	7	0	1	0
	4	8	3 5±2 0	4	0		
	5	13	5 0±1 3	4	0 3±0 3	4	2 5±2 5
	6	7	6 7±2 2	4	0	1	0
	7	18	4 3±1 3	6	0	2	0 5±0 5

crosses In others, no seeds were collected in covered and control-pollinated flowerheads, suggesting self-sterility and indicating that all seeds collected in crossed flowers were, in fact, hybrids Consequently, seeds of plants such as plants 6 and 7 of row F and plants 3 and 4 of row D were singled out for further examination in the greenhouse in the winter of 1994 and in the field in the summer of 1995

3 4 2 F1 hybrids

None of the many F1 hybrids tested in the greenhouse showed any extraordinary features resembling var *ethiopica* (Mills et al 1995) This observation, however, should be evaluated with caution, since at the time some of the characteristics (e g receptacle diameter) were not measured

Plant dimensions of the F1 hybrid plants grown in the field in the summer of 1995 were mostly intermediate between those of the parents, var *galamensis* (V-004) and *ethiopica* (V-001) (Table 6) It made little difference whether V-004 served as the female or as the male parent (Mills et al 1996) The most important finding was that the F1 hybrids flowered in summer at different intervals after transplanting, usually similar in length to that characteristic of one parent, namely var *galamensis* Plants of the other parent, var *ethiopica*,

Table 6 Phenological characteristics of plants of ssp *galamensis* var *galamensis* (accession V-004) and var *ethiopica* (accession V-001) and their hybrids grown in the field in 1995

Each value is a mean of 9-15 plants of the group Five branches, flowerheads and leaves were measured in each plant

Phenological parameter	V-004	V-001	V-004 x V-001
Plant height (cm)	57 1 b	110 5 a	71 1 b
Plant width (cm)	38 7 c	30 c	52 4 b
Height of first flower (cm)	22 0 a		23 6 a
No of primary branches	11 1 a		11 0 a
Length of 2ndary branches (cm)	23 7 b		40 5 a
No of 2ndary branches	3 5 a		4 6 a
Main stem diameter (mm)	13 9 a	14 2 a	17 6 a
2nd branch diameter (mm)	5 1 b		7 6 a
Leaf width (cm)	1 7 a	1 2 b	1 3 b
Leaf length (cm)	11 6 c	18 7 a	15 5 b
Leaf length/width	7 2 c	15 5 a	11 9 b
Receptacle diameter (mm)	10 9 b		15 2 a
Involucre length (mm)	13 7 a		16 0 b
Flower color *	2		3
Peduncle length (mm)	12 7		10 2
Flowering (days from sowing)	79		88

*

0 - white, 1 - light blue, 2 - blue, 3 - purple, 4 - dark purple

did not flower at all The different F1 hybrid lines of the same cross (V-004 x V-001) exhibited different characteristics (Table 7) According to leaf and flowerhead dimensions, F1h hybrids were closer to var *galamensis*,while D3h and F6h were closer to var *ethiopica* The former was excluded from the program Several plants of D3h and F6h hybrid lines, as well as a plant of the reciprocal cross (V-001 x V-004), were singled out for further selection The performance of one, D3h5/1, is described in Table 7 (This plant was chosen for further selection among other excelling plants mainly because of its resemblance to var *ethiopica*, however, it did not exhibit extraordinary characteristics as compared with the other selected plants)

Table 7 Phenological characteristics of F1 hybrids of ssp *galamensis* var *galamensis* (accession V-004) and var *ethiopica* (accession V-001) and a selected plant of the D3h group grown in the field in 1995

Each value is a mean of 3-5 plants of the group Five branches, flowerheads and leaves were measured in each plant

Phenological parameter	F1h	F6h	D3h	D3h 5/1
Plant height (cm)	57 5 b	86 0 a	64 6 b	78
Plant width (cm)	28 5 c	68 5 a	49 2 b	50
Height of first flower (cm)	13 5 a	29 1 b	22 8 b	22
No of primary branches	5 5 b	15 0 a	10 0 ab	12
Length of 2ndary branches (cm)	26 2 b	42 1 a	44 3 a	56
No of 2ndary branches	3 0 b	4 7 a	5 2 a	5 3
Main stem diameter (mm)	10 5 a	19 3 b	19 2 b	26
2nd branch diameter (mm)	5 5 b	7 6 a	8 3 b	7 8
Leaf width (cm)	1 15 b	1 25 b	1 49 a	1 62
Leaf length (cm)	9 07 c	15 7 b	18 4 a	18 3
Leaf length/width	7 95 b	13 7 a	12 07 a	11 3
Receptacle diameter (mm)	11 6 c	16 9 a	15 4 b	16 2
Involucre length (mm)	12 8 c	16 0 b	17 4 a	17 4
Flower color *	3	3	3	3
Peduncle length (mm)	12 1 a	10 5 a	9 4 a	9 0
Flowering (days from sowing)	75	75	86	88

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

3 4 3 F2 hybrids

Plants of the F2 hybrid lines started flowering after 11-13 weeks, while var *galamensis* (V-004) flowered after 10 weeks and var *ethiopica* did not flower at all (Table 8) In 1996 (as in the previous year), the hybrid between var *galamensis* and var *ethiopica* (V-004 x V-001) flowered earlier than the

reciprocal cross (V-001 x V-004), F1 and F2 In the F2 hybrids, as in the F1 hybrids (Mills et al 1995), leaf and flowerhead dimensions were intermediate between the two parents The flowerhead dimensions of the V-001 x V-004 plants were significantly smaller (by 2 5 mm) than those of the F1 plants In the V-004 x V-001 F2 hybrids, the degree of branching was similar to that of V-004 (Table 8), while in the V-001 x V-004 F2 hybrid it was less (no 7th level branches) Length of the various branches of V-004 x V-001 F2 hybrids was bigger than those of var *galamensis* (V-004) Differences were found between D3h F2 hybrid group and F6h F2 hybrid group and among plants within each of these selections (Table 9) D3h hybrid group had somewhat bigger flowerheads, bigger seeds and higher seed filling than F6h group (Table 9) but flowered nine days later on an average It seems, therefore, that D3h group is closer in characteristics to the male parent, var *ethiopica* and the group of F6h to the female parent, var *galamensis* Within the D3h group, descendants of D3h5/1 hybrid were taller plants and were more branched Within this group, a particular one plant, D3h5/1,5/7 excelled in branching (had 7 level branching as var *galamensis*) and thus in flowerhead production (flowerhead number was not determined) Beside having 19% seed filling, all other characteristics were similar to the mean of D3h5/1 hybrid plants (Table 9)

Table 8 Phenological characteristics of ssp *galamensis*, var *galamensis* (accession V-004) and F2 hybrids with ssp *galamensis*, var *ethiopica* (V-001) four months after transplanting in the field in March 1996

Each value is a mean of 10-30 plants of the group (in 3 blocks) Five branches, flowerheads and leaves were measured in each plant

Phenological parameter	V-004	V-001	V-004 x V-001 F2	V-001 x V-004 F2
Plant height (cm)	70 2 a	115 8 b	75 2 a	76 8 a
Height of first flower (cm)	9 8 a	-	11 4 a	14 3 a
Length of 2ndary branches (cm)	11 3 a	-	24 3 b	26 5 bc
Length of 3rd order branches (cm)	19 3 a		25 7 b	21 6 a
Length of 4th order branches (cm)	16 8 a		25 4 b	16 2 a
Length of 5th order branches (cm)	15 3 ab		17 0 b	13 7 a
Length of 6th order branches (cm)	13 8 a		12 6 a	11 5 a
Length of 7th order branches (cm)	11 3 a		3 0 b	-
Leaf width (cm)	1 3 bc	1 4 c	1 2 ab	1 1 a
Leaf length (cm)	9 7 a	18 0 d	12 2 b	12 0 b
Leaf length/width	7 51 a	14 13 d	10 5 b	11 59 c
Receptacle diameter (mm)	9 2 a	-	15 4 b	14 4 b
Involucre length (mm)	11 5 a	-	15 9 c	15 0 b
Ratio of full seeds (% of total)	13 a	-	45 b	29 ab
One thousand seed wt (g)	3 462 a	-	3 949 b	3 648 ab
Flower color *	2	-	3	3
Flowering (days from sowing)	73		77	86

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

Table 9 Phenological characteristics of the various F2 hybrids of ssp *galamensis* var *galamensis* (accession V-004) with ssp *galamensis* var *ethiopica* (V-001) and a selected plant of the D3h5/1 group four months after transplanting in the field in March 1996

Each plant population (e g , D3h 1/4) originated from a single F1 hybrid plant Each group of plant populations (e g , D3h) originated from a single plant of the original cross in 1994 Each value is a mean of 10-30 plants of the group (in 3 blocks) Five branches, flowerheads and leaves were measured in each plant Statistically significant difference (P<0 05) between means is designated in bold

Phenological parameter	D3h 1/4	D3h 5/1	D3h 1/1	D3h Mean	F6h 11/5	F6h 11/6	F6h 11/2	F6h Mean	D3h5/ 1 5/7
Plant height (cm)	66 5 a	83 0 b	69 0 a	72 4	79 5 a	80 5 a	74 2 a	78 7	103
Height of first flower (cm)	9 0 a	9 6 a	10 3 a	9 4	12 4 a	16 1 a	8 8 a	13 8	8
Length of 2ndary branches (cm)	25 6a	22 8 a	28 0 b	25 5	26 9 c	22 5 b	16 9 a	22 1	20 0
Leaf width (cm)	1 2 a	1 3 a	1 3 a	1 3	1 2 a	1 2 a	1 2 a	1 2	1 4
Leaf length (cm)	11 8 a	11 9 ab	13 0 b	12 2	12 4 b	11 3 a	14 0 c	12 5	9 72
Leaf length/width	9 8 a	9 0 a	7 81 a	9 38	10 3 a	9 42 a	11 67 b	10 4	6 94
Receptacle diameter (mm)	16 1 b	14 6 a	16 0 b	15 6	15 3 a	15 5 a	14 6 a	15 1	14 4
Involucre length (mm)	16 5 a	16 0 b	15 9 b	**16 2**	15 2 a	15 9 b	15 7 ab	**15 6**	15 4
1000 seed wt (g)	3 433 a	5 560 c	3 690 ab	**4 230**	3 158 a	3 807 b	3 864 b	**3 590**	5 53
Full seed (% of total)	57 b	34 a	59 b	50	36	38	49	40	19
Flower color *	3	3	3	3	3	3-4	3	3	3
Flowering (days from sowing)	84	90	96	**90**	78	86	78	**81**	90

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

3 4 4 F3 hybrids

Generally, F3 hybrids between var *galamensis* and var *ethiopica* showed phenological characteristics that were intermediate between those of the parents (Table 10) Again generally, hybrid lines of the cross V-001 x V-004 performed worse than lines of the cross V-004 x V-001 Var *galamensis* was not grown in this season (1996/97), but its characteristics are known from earlier observations (Mills et al 1995, Mills et al 1997) When var *ethiopica* served as the female parent, the F3 hybrids (V-001 x V-004) were closer in characteristics to it than when it served as the male parent (V-004 x V-001) (Table 10) This is reflected in the following characteristics height of the plant, leaf length/width and receptacle diameter Nevertheless other important characteristics such as number of capitula, seed weight and seed filling were rather better in V-004 x V-001 lines

Three F3 hybrid lines of the V-004 x V-001 cross were tested The characteristics of plants of the three lines varied greatly (Table 11) Line D3h5/1,5/7 excelled in number of flowerheads per plant and seed weight, line B7a/gh in wide capitula and early flowering, and line F6h 2/3f in seed filling The latter was generally inferior to the other two

It should be emphasized that in relation to var *ethiopica* (V-001), the hybrid plants exhibited superiority If we take line D3h, for example, it features a similar seed filling and seed weight to V-001 but has many more capitula and flowerheads earlier Different selections were made from the F3 hybrids (Mills et al 1998) The characteristics of one, the descendant of D3h, are presented in Table 13 This plant (D3h5/1,5/7, IV7a) excelled in high seed filling, early flowering and big capitula

3 4 5 F4 hybrids

Seeds collected from the selected F3 hybrids were tested in the field in the summer of 1998 In general, F4 lines of the cross V-004 x V-001 recalled the female parent var *galamensis* (V-004) more closely than the male parent var *ethiopica* (V-001) These plants had broader leaves, smaller receptacle, and more importantly, more capitula that flowered earlier, than F4 lines of the reciprocal cross (V-001 x V-004) The latter had characteristics similar to the male parent narrow leaves, larger receptacles and fewer capitula

Table 10 Phenological characteristics of ssp *galamensis* var *ethiopica* (accession V-001) and F3 hybrids of ssp *galamensis*, var *galamensis* (V-004) with ssp *galamensis* var *ethiopica* (accession V-001) and the reciprocal cross grown in the greenhouse in 1996/7

Each value is a mean of 11-36 plants of the group Five branches, flowerheads and leaves were measured in each plant

Phenological parameter	V-001	V-004 x V-001 F3	V-001 x V-004 F3
Plant height (cm)	83 b	78 4 b	80 8 b
Height of first flower (cm)	35 0 c	29 5 bc	26 1 ab
Number of primary branches	5 8 a	7 2 b	7 6 b
Leaf width (cm)	1 28 ab	1 57 c	1 35 b
Leaf length (cm)	11 9 c	11 9 c	11 0 b
Leaf length/width	9 62 c	7 74 a	8 39 b
Receptacle diameter (mm)	1 87 d	1 09 b	1 33 c
Involucre length (mm)	1 84 b	1 50 a	1 50 a
Ratio of full seeds (% of total)	44 5 b	43 4 ab	32 6 a
One thousand seed wt (g)	6 766 c	5 800 b	5 312 b
Number of capitula	47 a	240 b	198 b
Flowering (days from sowing)	104 b	95 a	95 a

Table 11 Phenological characteristics of F3 hybrids of ssp *galamensis* var *galamensis* (accession V-004) and var *ethiopica* (accession V-001) and a selected plant of the D3h5/1, 5/7 group grown in the greenhouse in 1996/7

Each value is a mean of 5-15 plants of the group Five branches, flowerheads and leaves were measured in each plant

Phenological parameter	V-004 x V-001 F3			
	D3h5/ 1,5/7	B7a/gh	F6h 2/3f	D3h5/1,5 /7, IV7a
Plant height	70 bc	90 de	73 bc	67
No of primary branches	6 3 ab	7 6 bc	7 7 bc	7
Leaf width (cm)	1 4 a	1 6 b	1 7 c	1 44
Leaf length (cm)	9 1 a	12 7 c	13 0 c	10 5
Leaf length/width	6 81 a	8 27 b	7 81 ab	7 29
Receptacle diameter (cm)	0 94 a	1 23 c	1 06 b	1 32
Involucre length (cm)	1 51 bcd	1 53 cd	1 47 ab	1 58
Number of capitula	311 d	259 cd	165 bc	224
Ratio of full seeds (% of total)	35 9 ab	27 2 a	44 4 b	71 4
One thousand seed wt (g)	6 287 cd	5 230 abc	6 063 cd	5 947
Flowering (days from sowing)	92 b	83 a	109 d	83

Table 12 Phenological characteristics of ssp *galamensis* var *galamensis* (accession V-004) and F4 hybrids of ssp *galamensis* var *galamensis* (accession V-004) with var *ethiopica* (accession V-001) and the reciprocal cross grown in the field in 1998

Each value is a mean of 15-70 plants of the group Five branches, flowerheads and leaves were measured in each plant

Phenological parameter	V-004	V-004 x V-001 F4	V-001 x V-004 F4
Plant height (cm)	57 3 a	53 0 a	49 4 a
Height of first flower (cm)	8 0 a	11 4 a	14 6 a
Leaf width (cm)	2 0 c	1 3 b	1 0 a
Leaf length (cm)	11 2 a	13 8 b	13 5 b
Leaf length/width	5 65 a	10 75 b	13 15 c
Receptacle diameter (mm)	8 4 a	11 3 b	12 2 c
Involucre length (mm)	12 1 a	13 7 b	13 4 ab
Number of capitula	43 7 c	20 6 b	7 1 a
Flowering (days from sowing)	100 a	105 a	118 b

Analyzing the performance of different hybrid lines of each type of cross showed that within the V-004 x V-001 group, D3h5/1,5/7IV7a hybrid plants excelled in early flowering (Table 13) and line F6h2/3fV5b in capitula production Within the V-001 x V-004 group, line III2b excelled in capitulum size and number of capitula but flowered late It should be noted that due to

Table 14 Phenological characteristics of ssp *galamensis* var *ethiopica* (accession V-001) and F4 hybrids of ssp *galamensis* var *galamensis* (V-004) with ssp *galamensis* var *ethiopica* (accession V-001) and the reciprocal cross grown in the greenhouse in 1997/8

Each value is a mean of 15-70 plants of the group Five branches, flowerheads and leaves were measured in each plant

Phenological parameter	V-001	V-004 x V-001 F4	V-001x V-004 F4
Plant height (cm)	107 c	87 a	97 b
Height of first flower (cm)	72 c	56 a	62 b
No of primary branches	6 1 a	12 1 b	11 0 b
Leaf width (cm)	2 5 c	3 3 a	2 7 b
Leaf length (cm)	16 6 a	15 3 b	14 4 c
Leaf length/width	6 91 c	4 78 a	5 55 b
Receptacle diameter (mm)	19 4 c	11 8 a	14 6 b
Involucre length (mm)	19 0 c	15 8 a	16 7 b
Full seeds (% of total)	62 a	25 b	21 b
One thousand seed wt (g)	6 79 b	5 51 a	5 77 a
Number of capitula	33 3 a	122 4 b	105 7 b
Flowering (days from sowing)	85 2 c	72 0 a	79 8 b

Analyzing the performance in the greenhouse of different hybrid lines of each type of cross showed that within the V-004 x V-001 group, D3h5/1,5/7IV7a hybrid lines excelled in early flowering (Table 15) and seed weight, line F6h2/3fV5b in seed filling, and line B7a/ghII5b in capitula production The latter, however produced few full seeds (7%) Within the V-001 x V-004 group, lines III6a and III1a excelled in capitula production but produced few full seeds and flowered late Of the plants grown in the greenhouse, only two plants belonging to the line of D3h5/1,5/7IV7a were selected for further testing Plant D3h5/1,5/7IV7a,20 excelled in early flowering and capitula production and plant D3h5/1,5/7IV7a,21 in big seeds and big capitulum (Table 15)

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somewhat lower than expected (about 35%), no difference was found between lines and seasons in this characteristic (Table 16)

In overall performance, the only outstanding characteristic of the hybrid lines is their flowering in summer They do not produce more flowerheads or seeds than either of the parent varieties

Table 16 Phenological characteristics of F5 hybrids of ssp *galamensis* var *galamensis* (accession V-004) with ssp *galamensis* var *ethiopica* (V-001) grown in the greenhouse in 1998/9

Each plant population (e g 20) originated from a single F4 hybrid plant Each value is a mean of 10-30 plants of the group (in 3 blocks) Five branches, flowerheads and leaves were measured in each plant Statistically significant difference (P<0 05) between means of each type of selected line is designated by different letters

	Winter/greenhouse		Summer/greenhouse		Summer/field	
Phenological parameter	D3h5/15/ 7IV7a, 20	D3h5/15/ 7IV7a 21	D3h5/15/ 7IV7a, 20	D3h5/15/ 7IV7a, 21	D3h5/15 /7IV7a, 20	D3h5/15 /7IV7a, 21
Plant height (cm)	65 2 a	76 2 b	74 4 a	82 0 b	68 0 a	86 7 b
Height of first flower (cm)	36 8 a	49 1 b	36 0 a	32 5 a	12 3 a	17 2 b
No of primary branches	9 4 a	6 2 b	10 4 a	8 3 b	10 6 a	10 2 a
Leaf width (cm)	2 2 a	2 3 b	1 6 a	1 6 a	1 3 a	1 6 b
Leaf length (cm)	8 7 a	9 5 b	7 9 a	9 3 b	10 7 a	13 3 b
Leaf length/width	4 04 a	4 21 b	5 14 a	6 14 b	8 4 a	8 5 a
Receptacle diameter (mm)	10 1 a	12 8 b	9 3 a	11 2 b	12 7 a	12 3 a
Involucre length (mm)	13 6 a	15 0 b	14 9 a	15 5 b	15 2 a	16 3 b
1000 seed wt (g)	4 688 a	5 933 b	n d	n d	n d	n d
Full seed (% of total)	27 a	42 b	15 a	37 b	n d	n d
Number of capitula	35 4 a	16 6 b	46 0 a	30 7 b	51 2 a	52 0 a
Flowering (days from sowing)	68 1 a	75 8 b	85 a	84 b	92 a	98 b
Oil (%)	30 4	30 8	n d	28 7	n d	n d

n d - not determined

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3 4 7 Summary of descendants of D3h line

Allowing for differences in growth habit between field and greenhouse, e g more vigorous growth in the greenhouse, the following statements may be made regarding the successive generations of our vernonia hybrids (Table 17) (1) Most characteristics are intermediate between the male parent, var *ethiopica* and the female parent var *galamensis* (2) Leaf length to leaf width ratio became smaller in the first generation but stabilized at about 8 5 (3) Receptacle diameter became smaller, the latter two observations could point to an increase in the expression of the female parent var *galamensis* and decrease in the expression of characteristics of the male parent var *ethiopica*, since the former has a small leaf length to width ratio and a wide receptacle (4) Flowering was consistently recorded about three months from sowing and was late by only 10-14 days as compared with var *galamensis* , this is one of the important characteristics of the hybrids (5) Number of flowerheads is low and quite variable This variability is probably due to agromanagement and soil and climatic conditions The small number of flowerheads produced is the major disadvantage of the hybrid lines developed through this program However, flowerhead production is similar to that of the parent lines (Table 17) It should be noted that plants of var *galamensis* can produce hundreds of heads per plant under certain agro-management and soil and climatic conditions, as was observed prior to initiation of this program in another growing location

Table 17 Phenological characteristics of F1-F5 hybrids of ssp *galamensis* var *galamensis* (accession V-004) with ssp *galamensis* var *ethiopica* (V-001)) (V-004 x V-001) grown in the field and greenhouse

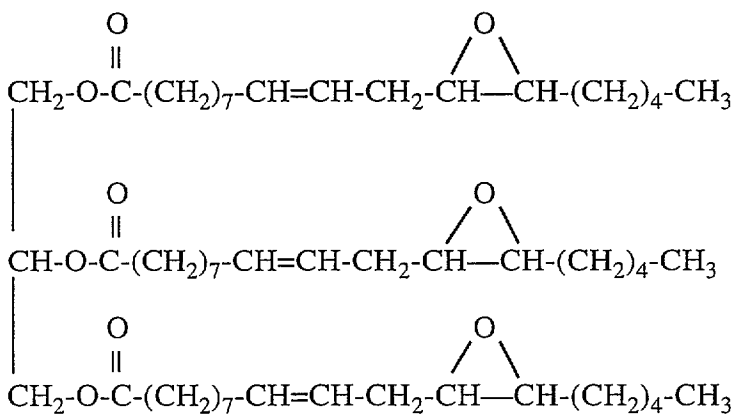
Phenological parameter	Parent				Descendants						
	ethiopica V-001	ethiopica V-001	galamensis V-004	galamensis V-004	F1 D3h	F2 D3h5/1	F3 D3h5/1 5/7	F4 D3h5/1 5/7IV7a	F4 D3h5/1 5/7IV7a	F5 D3h5/1 5/7IV7a, 20,21	F5 D3h5/1 5/7IV7a, 20,21
	Field	Greenhouse	Field	Greenhouse	Field	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse
Plant height (cm)	113	95	62	83	64 6	83	70	57	84 3	77	70 2
Height of first flower (cm)		54	15	48	22 8	9 6	-	7 8	51 8	14 8	43
No of primary branches		6	11	8	10	-	6 3	-	11 8	10 4	7 8
Leaf width (cm)	1 3	1 9	1 7	2 7	1 5	1 3	1 4	1 4	3 1	1 45	2 3
Leaf length (cm)	18 3	11 8	10 8	12 8	18 4	11 9	9 1	11 9	13 0	12 0	9 1
Leaf length/width	14 8	6 21	6 53	8 7	12 1	9 15	6 81	8 5	4 3	8 5	4 1
Receptacle diameter (mm)		19 1	9 5		15 4	14 6	9 4	11 8	12 1	12 5	11 5
Involucre length (mm)		18 7	12 4	15 9	17 4	16 0	15 1	13 3	17 3	15 8	14 3
1000 seed wt (g)		6 78	3 43		-	5 560	6 287	-	6 104	-	-
Full seed (% of total)		53	13		-	34	36	-	32	-	35
Number of capitula		40	44		-	-	311	15	95 5	52	26
Flowering (days from sowing)		95	84		96	90	92	94	65	95	72

3 5 Chemical transformation of *Vernonia galamensis* oil

3 5 1 Background

In the past few years there has been a growing consensus among the industrialized nations of the world regarding the need to find raw materials from sustainable and renewable resources *rather than from finite petroleum resources,* which are constantly being depleted as sources of energy and chemical feedstocks The chemistry objective of our AID-CDR project is to study the chemistry of the very special epoxy oil of vernonia plant and to synthesize novel products with potential industrial applications in order to develop *Vernonia galamensis* as a new industrial crop for semiarid zones

Today, commercial epoxy oils are synthesized from soybean or linseed oil by an industrial epoxidation process that triples or quadruples the price of the original oil In this type of application, vernonia oil (**I**) has a built-in advantage in that it is already epoxidized



Trivernolin (I)

Naturally functionalized triglyceride oils are prepared by nature with multiple chemical functionality and are thus a renewable resource, providing an alternative to petroleum as a chemical feedstock Vernonia oil is among a small group of naturally epoxidized triglyceride oils and has received considerable attention as its status nears that of a commercial material

Moreover, there is a growing realization that many biopolymers derived from agricultural feedstocks are rapidly biodegraded when composted and should thus be used to replace materials that are degraded slowly

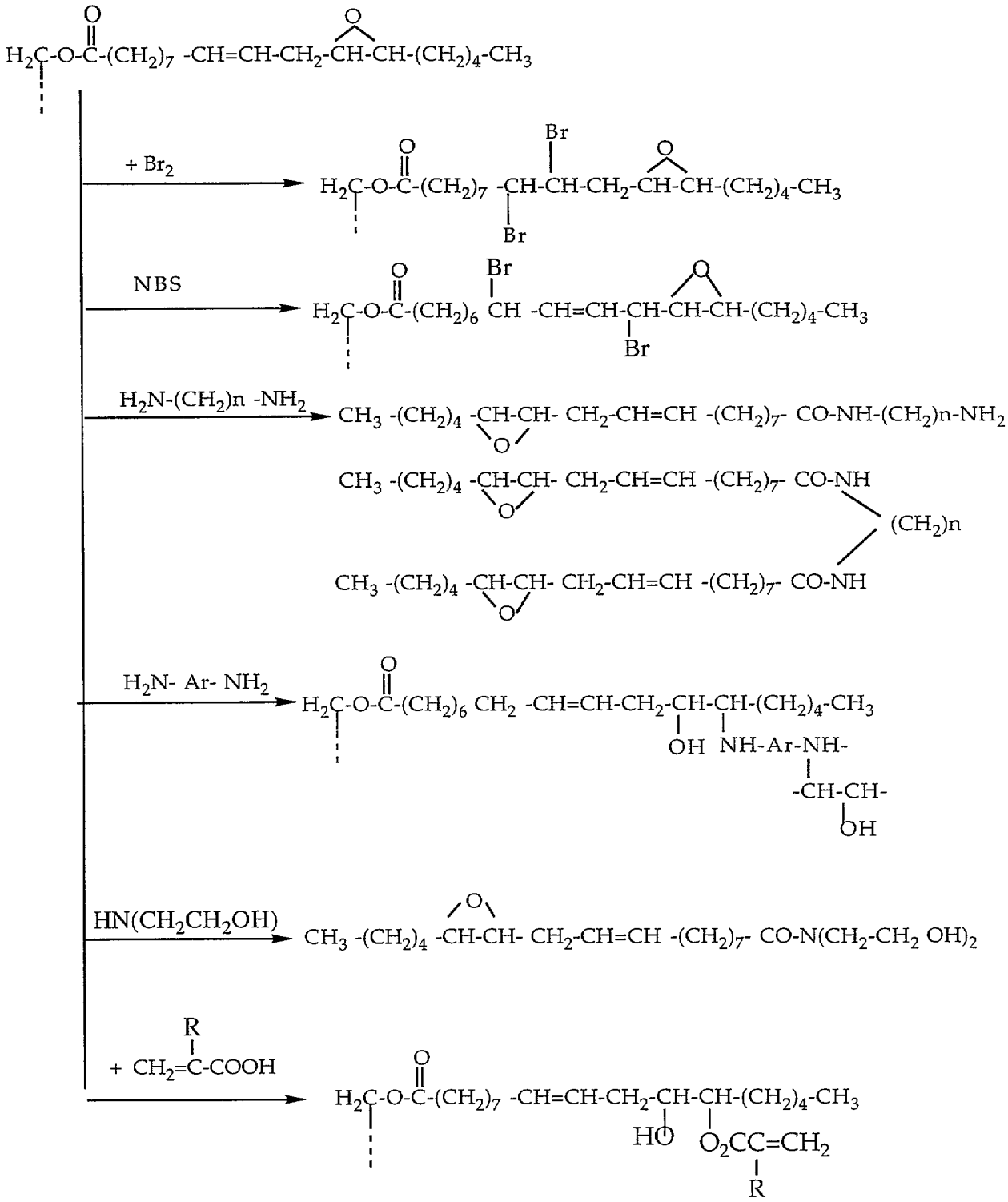
The timing is right for polymer materials and products designed and engineered from agricultural feedstocks to enter specific markets currently occupied by petroleum-based feedstocks Chemists must thus focus their attention on renewable resources and ensure that the demand for polymers in the 21st Century can be met by natural products

Most of the chemical research on vernonia oil has focused on its application as 1) a reactive diluent for epoxy coatings (Dirlikov, 1990), or 2) a monomer in the synthesis of polymers (Sperling, 1983, Barret, 1993) Ayoride and co-workers (Ayoride *et al*, 1988, 1989, 1990) have made progress in transformation of the oil into key industrial raw materials, e g a series of dibasic acids have been prepared

In the first stage of our research, we studied the chemical composition of the Israeli vernonia oil We examined Soxhlet and room temperature extraction of ground seeds of accession V-001 of ssp *galamensis* var *ethiopica* grown in the field or greenhouse It was found that Soxhlet extraction yielded somewhat more oil than stirring ground vernonia seed with hexane at room temperature The oil obtained at room temperature contained a lower concentration of free fatty acids and the oxirane value was higher Another method involving lipase deactivation followed by extraction of the oil at room temperature was tried We obtained 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%), approximately 0 85-2 16% of free fatty acids (FFA) Extracting the oil at room temperature assured a relatively high (80%) content of vernolic acid

Our main task in the framework of the AID-CDR project was to explore ways of utilizing this highly attractive industrial raw material Specifically, our task was to prepare new derivatives of vernonia oil as intermediates for the chemical industry by exploiting the unique structure of the vernonia molecule

Scheme 1 summarizes the main directions we took



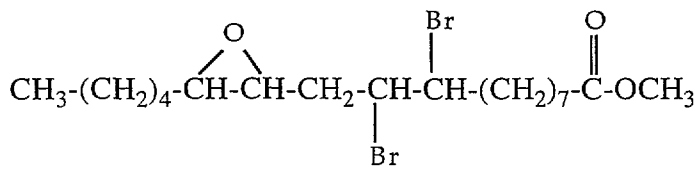
Scheme 1

3 5 2 Bromination

Bromination reactions were performed on the methyl ester of vernolic acid, on the vernonia oil itself, and on the 1,3-divernolin Bromo derivatives were prepared by addition and allylic bromination

Bromination of methyl vernolate

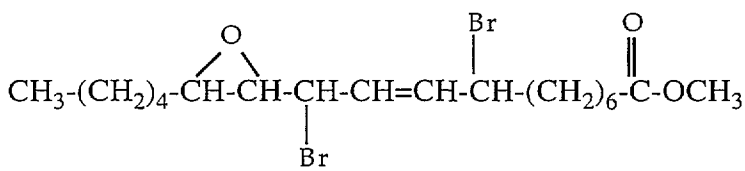
The bromination of the 9,10 double bond of the methyl vernolate was carried out at different temperatures in various solvents (carbon tetrachloride, dichloromethane, and acetonitrile) The best results were obtained with carbon tetrachloride at 0°C The pure dibromo methyl vernolate was isolated by column chromatography



II

From the ^{1}H-NMR spectrum 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 (II) to be a *threo* isomer However, in the ^{13}C-NMR spectrum there are low intensity signals (15-20%) that correspond to the *erythro* isomer

We also carried out bis-allylic bromination of methyl vernolate with N-bromosuccinimide (NBS) under different conditions to obtain a complex mixture of bromo compounds



III

The product (III) was separated by column chromatography and contained only 60% of the main substance Signals in the ^{1}H-NMR spectrum were observed at 3 02 (2H, epoxy group), 4 49 and 4 67 (2H, CHBr group), and 5 90 (2H, olefinic protons) d ppm

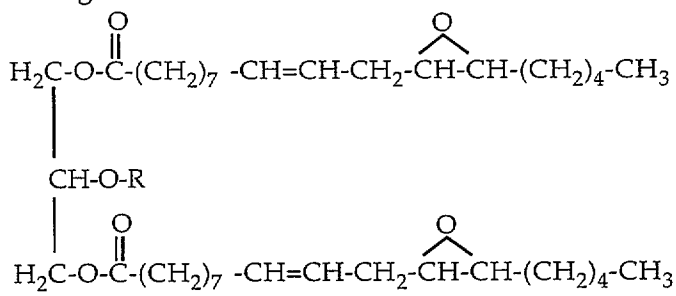
Bromination of vernonia oil

Bromination of the oil was carried out with bromine or with the bromine complexes dioxane dibromide ($C_4H_8O_2Br_2$) or pyridinium hydrobromide perbromide ($C_5H_5NHBr_3$) The reactions were carried out over a range of

temperatures in different solvents (carbon tetrachloride and dichloromethane) Since the predominant triglyceride is trivernolin (**I**), all calculations were performed on the basis of this compound The TLC and NMR spectra show that bromination of the oil gave rise to complex mixtures of bromocompounds in all cases tested We can, however, assume that the structure of the main compound in each mixture corresponds to the addition of bromine to the double bond of the oil The greatest quantity of oxirane groups was obtained when bromination was carried out with pyridinium hydrobromide perbromide From all these experiments it is evident that the oxirane groups in the triglycerides are more labile in bromination reactions than the glycerides in methyl vernolate

Bromination of 1,3-divernolin

1,3-divernolin (**IV**) was obtained from vernonia oil in hexane by using a crude lipase extract from *V galamensis* seeds



R= H, CH_3CO **IV**

The ^{13}C-NMR and IR spectra confirmed the structure of 1,3-divernolin (R=H)

Bromination of 1,3-divernolin was performed with pyridinium bromide perbromide, since we had found in previous experiments that this brominating agent that does not have a significant effect on the epoxy group in CCl_4 at room temperature As in the bromination of vernonia oil, a complex mixture of brominated compounds was formed In addition to the bromination of the double bonds, changes occurred in the signals of the epoxy protons they appeared as two small multiplets

Bromination of 1,3-divernolin-2 acetate

Pyridinium bromide perbromide was again used as the bromination reagent for the bromination of 1,3-divernolin-2-acetate at 40°C The crude product was purified by column chromatography to give the tetrabromo derivative of divernolin acetate In the IR spectrum we still could see the epoxy group The ^{1}H-NMR spectrum also showed the 4 protons of the epoxy group and the CH-Br protons

3 5 3 Aminolysis of vernonia oil

Our interest in developing industrial products from *Vernonia galamensis* led us to produce fatty mono- and bisamides from vernonia oil

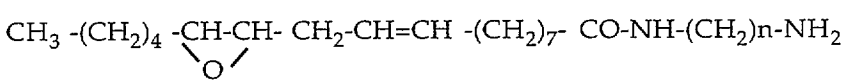
Fatty amides, compounds that exhibit low reactivity and high thermal stability, have important applications in the chemical industry in paper coatings, printing ink, and antistatic and anti-block additives for polyethylene films Fatty bisamides, which have a higher lubricating efficiency than primary amides, are used as slip agents, they also find application as corrosion inhibitors In industry, mono-substituted amides (RCONHR) are typically produced either from purified fatty acids and primary amines at temperatures of 140-170°C under moderate pressure, or from a fatty acyl chloride and an amine or fatty esters and an amine with sodium methoxide as the catalyst (Jordan & Port 1961)

Reaction of vernonia oil with aliphatic diamines
The group of Ayorinde (Bryant et al 1993) reported the synthesis of novel epoxy-containing fatty amides from vernonia oil by reaction of primary amines with the naturally occurring epoxy triglyceride under mild conditions Grinberg and co-workers (1993, 1994) synthesized N,N'-ethylenebis(vernolamide) by the direct amidation of vernonia oil with aliphatic diamines

In this part of our research, we studied the reaction of vernonia oil with aliphatic diamines

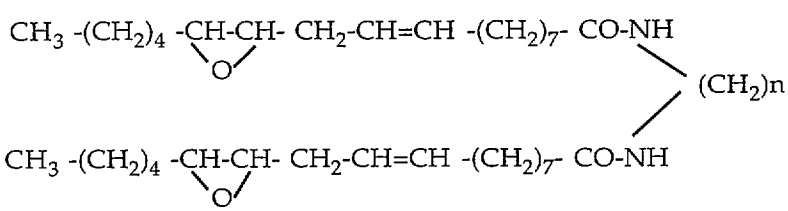
vernonia oil + diamine → monoamidoamine + diamide

This involved isolation and characterization of the intermediate—the monoamidoamine of vernolic acid (**V**)



V

and of the final product—N,N'-alkylbis (vernolamide) (**VI**)



VI

Diepoxy diamides contain two epoxy groups in the molecule, they can therefore be used as a new class of reactive difunctional monomers with readily available amide blocks We have developed improved procedures to obtain high yields of both intermediate and product Using these epoxy diamides as reactive monomers and study of their polymerization is one of our future tasks

In the framework of the cooperation with Kenya, Ms Mary Warnira prepared an M Sc thesis on the reaction of vernonia oil with aliphatic diamines A study was made of the effect of different parameters, such as molar ratio oil diamine, temperature, and solvent, on the course of the reaction The reaction of the oil with 1,4-diaminobutane was chosen as model reaction The progress of the reaction, in terms of reactant disappearance and the appearance of the two products, the intermediate amidoamine and the final diamide, was monitored by TLC, GC and HPLC The products were separated and characterized spectroscopically (IR, NMR)

Table 18 Effect of the molar ratio of vernonia oil (VO) to diamine on the yield of the products at 70°C without solvent

Molar ratio	After 0 5 h		After 1 h		After 2 h	
VO diamine	Monoamido-amine	Diamide	Monoamido-amine	Diamide	Monoamido-amine	Diamide
1 1	2 38	3 4	-	6 3	2 2	13 5
1 2	24 9	4 2	22 2	10 27	31 09	23 42
1 3	28 7	10 2	34 7	17 3	36 62	26 3
1 5	61 22	5 56	72 33	13 37	56 41	20 4

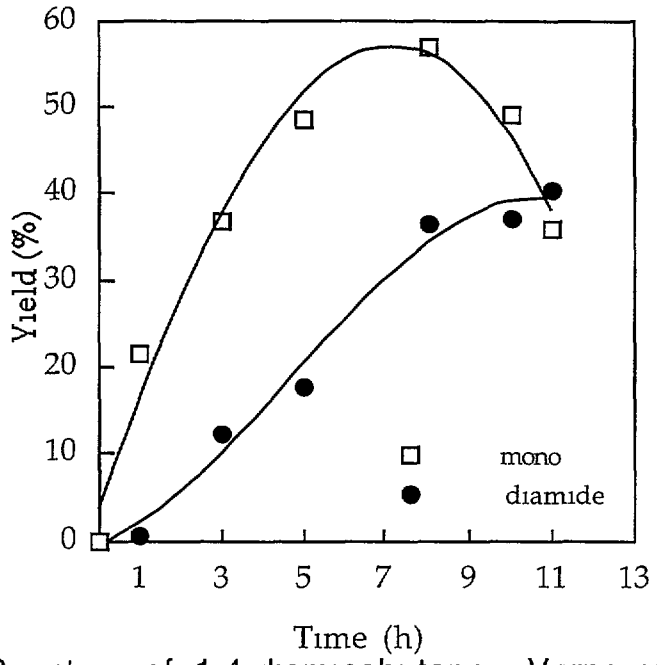
VO = vernonia oil

As we can see from Table 18, the yield of monoamidoamine increased with increasing molar ratio, from equimolar amounts up to fivefold excess of diamine For diamide formation, there was an increase in yield with molar ratio up to 1 3 A higher excess of diamine did not increase the yield of the products, probably because side reactions occurred with opening of the epoxy ring A rubber-like product was obtained at an oil diamine molar ratio of 1 10 at 70°C after 1 h

After determining that monoamidoamine and diamide were indeed the products of the reaction of vernonia oil and diamine, we studied the profile of the reaction

The Reaction Profile

As expected, the rate of formation of amidoamine is faster at the beginning of the reaction, reaches a maximum after about 6 h, and then starts to decrease, while the rate of formation of diamide is slow at the beginning and then increases with time—a profile characteristic for a consecutive reaction



Reaction of 1,4-diaminobutane +Vernonia oil in 2ml- $CHCL_3$ at the refluxing Temp of $CHCL_3$
Molar ratio 1 3 Vo/diamine

To facilitate analysis and characterization of the amidation products of vernonia oil, we first performed aminolysis of methyl vernolate, as follows

Reaction of methyl vernolate with aliphatic diamines
We reacted the methyl ester of vernonia oil with aliphatic diamines using an ester diamine molar ratio of 2 1 The reaction was carried out at 60°C for about 6 hours in the presence of a 2N solution of sodium methoxide in methanol The reaction was followed by TLC This is a consecutive substitution reaction, the monoamidoamine of vernolic acid is formed in the first stage of the amination reaction and the diamide N,N'- alkylene bis(vernolamide) in the second stage We used the different solubility properties in acetone or methanol of the mono- and divernolamides to separate them from the reaction mixtures The diamide is a white powder with a 13 7% content of epoxy groups and M P of 126-128°C

An HPLC method based on a C-18 reverse phase column was also used to determine the purity of the obtained diamide IR and NMR spectroscopy were used to identify the structure of N,N'- alkylene bis(vernolamide)

3 5 4 Reaction of vernonia oil with aromatic diamines

Sperling and Manson (1983) reported the synthesis of interpenetrating polymer networks on the basis of cured elastomers prepared from vernonia oil and dibasic acids Dirlikov (1990) and Frischinger*et al* (1992) have investigated the

preparation and properties of IPNs prepared from commercial epoxy resins (EPON 825) cross-linked with aromatic diamines (4,4'diaminodiphenyl sulfone, 4,4'-methylenedianiline) and from elastomers based on vernonia oil cross-linked with the same diamines for coating applications The modifed commercial rigid epoxy resin showed improved toughness and water resistance

After we performed the reactions of vernonia oil with aliphatic diamines, we were interested to study the reaction with aromatic diamines with a view to synthesizing elastomers, characterizing them, studying their properties, and utilizing them to prepare IPNs with commercial epoxy resin cross-linked with a cross-linker different from aromatic diamine

Epoxy resins cured with aromatic diamines are strong and exhibit good chemical and electrical resistance and excellent adhesive properties, they can thus be used in numerous technical applications (Barclay 1992, Grillet 1991, Gupta 1991) In addition, insertion of aromatic fragments into the polymer matrix could increase its thermal stability Under conditions under which it is normally possible to cure epoxy resins based on the diglycidyl ether of bisphenol-A (Gupta, 1991), there was no reaction with vernonia oil This could be because the epoxy groups of vernonia oil are in the middle of the chain and are thus less reactive than the terminal epoxy groups of epoxy resins

Since it is known that the reaction of epoxy groups with aromatic amines may be accelerated by compounds containing an active hydrogen (acids, alcohols etc) (W G Potter, 1970), we performed the reaction of vernonia oil with *m*-phenylene diamine in the presence of *p*-toluene sulfuric acid (*p*-TSA) as a catalyst The influence of molar ratio of epoxy groups to amine groups and concentration of *p*-TSA on gel time are shown in Table 19

Table 19 Reaction of vernonia oil with *m*-PDA at 100°C in the presence of *p*-TSA

Molar ratio epoxy/ amine	Concentration of *p*-TSA, mol [a]	Gel-time (h)	External appearance	Gel fraction[b] (%)	N(%) in gel fraction
1 1	0 25	2	gel	65	2 6
1 5 1	0 25	3	gel	55	2 7
3 1	0 25	a viscous liquid obtained after 5 h	-	-	

[a]Amount of *p*-TSA is expressed as moles per moleof triglyceride-containing epoxy group
[b]Gel fraction size is determined in DMF after extraction of the polymer in boiling DMF, ethanol water

From Table 19 we can see that gel time depends mainly on the molar ratio of the reacting groups At epoxy amine molar ratios of 1 1 and 1 5 1, the gel time is 2-3 h At molar ratio 3 1, only a viscous liquid is obtained Gel fraction content was close to the theoretical value (~70%) Nitrogen content in cured polymers

(2 6-2 7%) was close to the theoretical amount calculated for epoxy NH_2 molar ratio of 1 5 1 (2 7%) No sulfur was found in the obtained polymers

The ability of *p*-TSA to catalyze the cross-linking reaction was also investigated with other aromatic diamines (Table 20)

Table 20 Reaction of vernonia oil with DDS and DDO in the presence of 0 5 mol p-TSA and at epoxy amine molar ratio of 1 1

Diamine	Temp (°C)	Gel time (min)	External appearance	Gel fraction in polymer (%)	N[a] (%)	S[a] (%)
	150	10	gel	45	2 25	2 7
DDS	120	120	gel	50	2 1	2 0
	100	390	viscous liquid	-	-	-
	150	5	gel	70	2 62	0
DDE	120	40	gel	52	2 45	0
	100	150	sticky soft product	-	-	-

[a]The calculated contents of N,% and S,% for the polymer with an epoxy amine molar ratio of 1 5 1 are for DDS, N = 2 31% and S = 2 73% , for DDE, N = 2 45%
DDS- 4,4 -diaminodiphenyl sulfone, DDE-4 4 -diaminodiphenyl ether

Reactions were carried out for an epoxy to NH_2 molar ratio of 1 1 and concentration of *p*-TSA of 0 5 mol From Table 20 we can see that with DDS and DDE at 100°C, cured polymers were not obtained The times of gelation at 120°C and 150°C were of the order of 1-2 h and 5-10 minutes, respectively, for both DDS and DDE A comparison of the gel times of the polymers obtained from DDS or DDE with *m*-PDA at 100°C (Table 19) reveals that *m*-PDA is more reactive These differences in the gel time may be attributable to sterical difficulties due to the larger sizes of the DDS or DDE molecules The amounts of nitrogen and sulfur in the polymers obtained were close to the theoretical amounts for polymers at an epoxy amine molar ratio of 1 5 1 Polymers cured with DDS and DDE have gel fraction sizes of 45-70%

The structure of the cured polymers was investigated by IR spectroscopy The IR-spectra of all the cured polymers show that the absorption characteristic of epoxy at 820 and 840 cm^{-1} is absent, while carbonyl absorption at 1730 cm^{-1} remains New absorption peaks at 1500 and 1580 cm^{-1} indicate the presence of an aromatic ring New peaks at 3350 and ~3500 cm^{-1} can be attributed to NH and OH groups, respectively These changes indicate that aromatic diamine reacts with the epoxy group and that the epoxy ring is opened with the formation of NH and OH groups

The thermal behavior of the cross-linked polymers obtained was studied by thermogravimetric analysis (TGA) As a criterion of thermal stability of these products, we have chosen the following temperatures T_{start} - start of degradation, T_{10} -10% weight loss,T_{50} - 50% weight loss The results (Table 21) show that, as expected, all the polymers are more thermostable than original vernonia oil The structure of the diamines influences mainly the value of the T_{start} of the polymers The diamines can be arranged in the following series as a function of T_{start} DDE > DDS > *m*-PDA

Table 21 Results of TGA

Product	T_{start}, °C	T_{10}, °C	$T5_0$, °C
Vernonia oil	220	290	360
Polymer from vernonia oil with *m*-PDA	290	360	440
Polymer from vernonia oil with DDS	310	375	430
Polymer from vernonia oil with DDE	350	375	460

It should be noted that the cured polyamines of vernonia oil with aromatic diamines reported by Dirlikov (1990) and Frischinger (1993) were prepared under far more drastic conditions (180°C for 40 h) than in our synthesis (100°C for 1-3 h)

Interpenetrating polymer networks

The interaction of aromatic diamines with vernonia oil resulted in the production of a series of new rubber-like polymers One of the polyamines obtained from VO and *m*-PDA was used to modify commercial epoxy resin (Epicote 828) Our method of preparing the IPNs was based on polymerization of the commercial epoxy resin in the presence of curing accelerator [2,4,6-tris(dimethyl-aminomethyl)phenol - DMP-30] and poly-condensation of VO with *m*-PDA The preparation of IPNs requires the use of two liquid prepolymers, which can then be cured by post-polymerization

In addition to curing VO with *m*-PDA, we also investigated the conditions for preparing liquid prepolymers from these reagents Obviously, such prepolymers can be obtained beyond gelation

The results of our study of the reaction of VO with *m*-PDA at 100°C in the presence of 0 1 or 0 05 mol *p*-TSA and at different molar ratios of epoxy NH_2 (1 5 1, 1 1 and 1 1 5) are shown in Table 22 The reaction course was evaluated by following the changes occurring in the epoxy groups in the reaction mixture and the polymer gelation times Epoxy group content (in the presence of an amine group) was determined according to the method of Bel (1970)

Table 22 Preparation of prepolymers of vernonia oil with *m*-PDA at 100°C

Molar ratio epoxy /amine	*p*-TSA, mol	Conversion of epoxy-groups[a], %	Gel time, h
1 1	0 05	61	[b]
	0 1	88	5
1 1 5	0 05	86	5
	0 1	93	4
1 5 1	0 05	35	[b]
	0 1	50	6

[a] After 4 h, before appearance of gel
[b] No gel was observed after 7 h

The course of the reaction was evaluated by following changes in the epoxy groups in the reaction mixture and the gelation time of the polymer

From Table 22 we can see that the conversion of the epoxy group depends mainly on the molar ratio of the reacting groups However the gel time is determined mainly by the amount of *p*-TSA The 0 05 mol concentration of *p* -TSA decreases the time of gelation when compared with the 0 1 mol concentration

Prepolymers obtained with an epoxy NH_2 molar ratio of 1 1 and 1 5 1 in 4 h were viscous liquids Other prepolymers (epoxy NH_2 molar ratio 1 1 5) obtained in 4 h were close to gel formation Prepolymer obtained at epoxy NH2 molar ratio 1 1 was chosen to prepare the IPNs, because this prepolymer did not give gel in 2 h and showed a high conversion of epoxy groups (88%)

Similarly, liquid viscous prepolymers were obtained from epoxidized soybean oil (epoxol) containing 17% epoxy groups with *m*-PDA (epoxy NH_2 molar ratio 1 1, *p*-TSA0 1 mol, 100°) However, gelation in this case occurred faster than with VO, so the reaction with epoxol proceeded for 1 hour

Formulations were prepared by adding prepolymers of VO with *m*-PDA (or epoxol with *m*-PDA) to a mixture of Epicote 828 with 4% wt of accelerator These formulations were mixed at room temperature to produce homogeneous transparent mixtures The transparency of the mixtures obtained points to compatibility between the epoxy resin (Epicote 828) and the respective prepolymer

After degassing with a vacuum pump, the mixtures were poured into the Teflon forming molds, which were heated at 80°C for 2 h and then at 160 °C for 4 h The following combinations of epoxy resin/prepolymer (VO or epoxol with *m*-PDA) were prepared 100/0, 50/50 and 0/100% wt The mechanical properties of the castings obtained are shown in Table 23

Table 23 Mechanical properties of castings

Casting composition, epoxy resin prepolymer, % wt	Tensile strength, MPa	Elongation, %	Young's module, MPa
100/0	86	5 6	2206
0/100 (VO with *m*-PDA)	0 5	18 3	4 4
50/50 (VO with *m*-PDA)	2 2	61	5 4
0/100 (epoxol with *m*-PDA)	2 5	40	50
50/50 (epoxol with *m*-PDA)	35	11	562

From Table 23 one can see that the original cured epoxy resin is tough and brittle, while the neat vernonia oil polyamine is a very weak, rubbery material The mechanical properties of this polyamine are similar to those of vernonia oil-sebacic acid polyester (tensile strength 0 26 MPa, elongation 17% and modulus 1 7 MPa) (Sperling, 1990) IPN containing 50% of vernonia oil polyamine is a reinforced elastomer with an elongation three times greater than that of neat polyamine vernonia oil Neat epoxidized soybean oil (epoxol) polyamine is stronger than polyamine vernonia oil IPN containing 50% of epoxol polyamine is a tough material (tensile strength 35 MPa) with a fairly high Young's modulus (562 Mpa)

The mechanical properties of known IPNs obtained on the basis of elastomers from natural oils are shown in Table 24 In order to be able to compare our IPNs with known IPNs, we included in the table those IPNs in which the amount of elastomer was close to that in ours

Table 24 Mechanical properties of known IPNs

IPN, ratio of polymers, % wt	Tensile strength, MPa	Elongation, %	Modulus, MPa	Reference
VO-sebacic polyester/ PET = 50/50	6 03	46 7	25 8	L W Barrett J Appl Pol Sci 48, 953 (1993)
Castor oil polyurethane/PET = 50/50	16	1	-	L W Barrett JAOCS, 70, 523 (1993)
Castor oil polyuretane/ PSN = 40/60	23	100	-	L H Sperling JAOCS, 60, 1887, 1983
Epoxidized crambe oil/ PSN = 40/60	15	63	76	
Epoxidized linseed oil/ PSN = 40/60	18	37	51	
Epoxidized lesquerella oil/PSN = 40/60	13	56	21	
Castor oil polyurethane/PMM = 50/50	25	~180	-	L Zhang, H Ding J App Pol Sci 64, 1393 (1997)

PET= poly(ethylene terephthalate), PSN = polystyrene network,
PMM= poly(methyl methacrylate)

Examination of the data in Tables 23 and 24 shows that IPNs containing 50% of vernonia oil polyamine elastomer (Table 23) had the lowest values for the tensile strength and Young's module, while the elongation was high (61%) The IPNs containing 50% epoxol polyamine were tougher (35 MPa) and had a much higher modulus (562 MPa) than the known IPNs

The results obtained demonstrate the possibility of preparing new rubber-like polymers from vernonia oil and aromatic diamines The curing conditions required for these polymers (100-120°C, 2 h) were milder than those used for obtaining prepolymers from vernonia oil and dibasic acids (140°C, 7 h) (Alofabi, 1989) In terms of their thermostability and elastomeric properties as well as the simplicity of synthesis, the polymers derived from vernonia oil/aromatic diamine and epoxol/aromatic diamine represent a promising pathway to the synthesis of new interpenetrating polymer networks

3 5 5 Surface active compounds based on vernonia oil

The next new class of chemical derivatives we developed in the framework of the AID-CDR project are the vernonia-based surfactants Surface-active compounds are probably among the most versatile chemicals available They are used in the manufacture of a wide range of consumer products, including chemicals, pharmaceuticals, soaps and detergents Growing demand for surfactants worldwide has spurred a search for new raw materials for their production Nowadays consumer demand that surfactants be produced using environmentally friendly and non-toxic molecules based on renewable resources, moreover, the cost of production must be competitive Vernonia could prove to be an appropriate material for this purpose

Vernolic acid salts

Carboxylate together with sulfonate, sulfate and phosphate are the polar solubilizing groups found in anionic surfactants Soaps represent most of the commercial carboxylates The general structure of soap is $RCOO^-M^+$, where R is a straight chain hydrocarbon in the C_9-C_{21} range and M^+ is a metal or ammonium ion The neutralization of fatty acids with a base or an amine is a common procedure for the production of soluble fatty acids (Pryde 1979)

$$CH_3\text{-}(CH_2)_4\text{-}\overset{\diagup O \diagdown}{CH\text{-}CH}\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COO^-\ M^+$$

$$M = Na, K, NH_4$$

Salts of vernolic acid and dihydroxy vernolic acid were prepared directly by saponification of vernonia oil or of the methyl esters (obtained from transesterification of the oil) with sodium hydroxide in absolute methanol, as well as from the corresponding acids and potassium hydroxide in absolute methanol or sodium carbonate in a hexane/water mixture In all these reactions, some degree of opening of the epoxy ring occurred

Recrystallization of the salts was performed from a ~5% aqueous solution with an acetone/hexane/ether mixture, or from a methanol solution with an

ether/hexane mixture The former salt was obtained in very poor yield (less than 1%) because of poor precipitation of a dilute transparent gel By precipitation from methanol, a voluminous white suspension was obtained, this gave a reasonable yield of a white powder of salts when filtered and dried A decrease in the epoxy content and the appearance of new bonds at 1083 and 1736 cm^{-1} were evident in the spectrum of the salts

The bands at 1083 and 1736 cm^{-1} (as shoulders) in the IR spectrum were also visible in the spectrum of the mixture of fatty acids obtained both from vernonia oil and from the salts It is likely that these bands are related to estolide formation Estolides are formed when the carboxylic acid group of one fatty acid forms an ester bond with either a hydroxyl group or a double bond on the carbon chain of a second fatty acid (Erhan et al 1996) The band at 1083 cm^{1} pertains to the C-O stretching of secondary alcohols, and the band at 1736 cm^{1} to the carbonyl stretching of estolides (the absorbance of estolide carbonyl is 1737 cm^{-1} (Erhan et al 1996)

The data reported in the literature (Markley 1961) testify that "highly pure neutral metallic salts, especially of the long-chain fatty acids, are exceedingly difficult to prepare because of the tendency of the precipitated salt to include reaction reagents, to form hydrates or retain water and form solvates with organic solvents The salts of the unsaturated acids are subject to auto-oxidation" Preparation, isolation and cleaning of vernolic salts are additionally difficult because of the presence of the oxirane ring

Vernolic diethanolamide

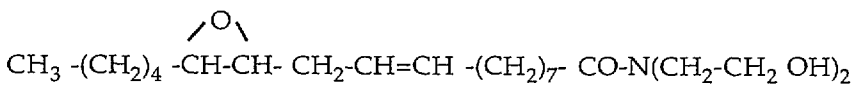
Secondary and tertiary alkanolamides are more commonly known as

monoalkanolamides $RCONH(CH_2)_y(CHCH_3)_2OH$

or

dialkanolamides $RCON[(CH_2)_y(CHCH_3)_2OH]_2$

Diethanolamides find application in liquid dishwashing detergents, shampoos, lubricating oils and dry cleaning detergents, as well as in textile processing



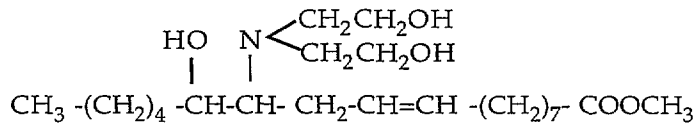
N,N-bis(2-hydroxyethyl) vernolamide

Vernolic diethanolamide was prepared from vernonia oil or the corresponding methyl esters and diethanolamine in the presence of sodium methoxide as catalyst The reaction proceded with retention of the epoxy ring (oxirane oxygen titration, IR and NMR spectra), the yield usually being over 90%

High purity N,N-bis(2-hydroxyethyl) vernolamide was isolated by column chromatography with 75 8% yield The IR spectra of vernolic diethanolamides are characterized by bands at 823 and 857 cm^{-1} (epoxy absorption), a split band at 1050 and 1070 cm^{1} (C-O stretching of ethanolic groups) and at 1620 cm^{1} (amide carbonyl stretching), and a broad band with maximum at 3388 cm^{1} (OH stretching) The spectra of mixed vernolic diethanolamides obtained from oil

or esters were identical They differed from the spectra of N,N-bis(2-hydroxyethyl) vernolamide purified by column chromatography in terms of impurities at the ester carbonyl stretching at 1741 cm^{-1} and the intensity of the epoxy absorption band

When vernonia oil or methyl vernolate were reacted with diethanolamine in the presence of an acidic catalyst, opening of the epoxy ring occurred and the following product was obtained



The compounds obtained were characterized by IR, ^1H and ^{13}C NMR

The properties of the new substances (solubility in water, critical micelle concentration and foaming properties) indicate that vernolic salts and diethanolamides are good candidates for a large range of surface active compounds (detergents, thickening agents)

The solubility of vernolic derivatives increases in the order

methyl esters (E)<acids (Ac)<amides (Am) <salts (S)

Introduction of polar groups in the surfactant alkyl chain results in considerable increases in the solubility of surfactants (Malik and Chand 1966, Ross and Miles 1941), thus, in water the solubility of potassium 9,10-dihydroxystrearate is superior to that of potassium stearate (Markley 1961) On the other hand, the lower the purity of fatty alkanolamides, the higher their solubility (Schick 1959) The lower solubility of amide Am_1 (obtained from methyl vernolate) as compared with mixed amides Am_2 (obtained from vernonia oil) supports the higher purity of amide Am_1

The critical micelle concentration (CMC) of our amides shows good correlation with those described in the literature (Malik and Chand 1966) and obtained by using similar reaction conditions

The foaming properties of two vernolic derivatives (anionic and nonionic) were determined by the standard method in both pure (deionized) water and hard water The data correlate with those described in the literature In pure water, vernolic salts RCOOM (R= vernolyl, M=Na, K, NH_4) produced a more abundant and more stable foam that the vernolic diethanolamides $RCON(CH_2CH_2OH)_2$

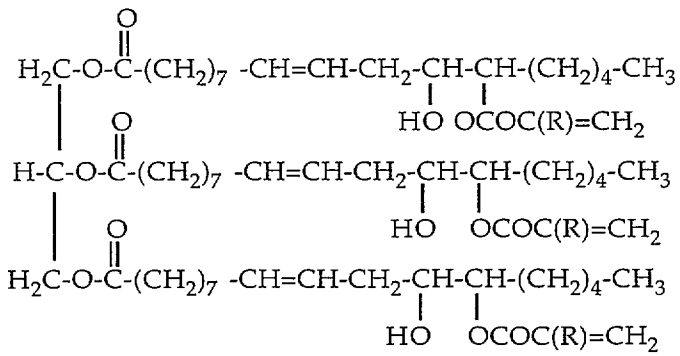
3 5 6 Synthesis of new acrylates and methacrylates based on vernonia oil

Another phase of our study on the synthetic applications of vernonia oil was the preparation of new polyfunctional acrylates Our reason for attempting this study was the belief that the synthesis of new acrylates and methacrylates based

on vernonia oil would contribute to the chemistry of vernonia oil, and would also represent an important step forward towards developing *Vernonia galamensis* as a new industrial crop through the synthesis of novel products with potential applications in industry These new acrylates can be used to synthesize polymers by an energy-efficient and pollution-free method Multifunctional acrylates (MFAs), usually obtained from petrochemicals, are among the most important of the classes of reactive monomers that can easily be photopolymerized by UV radiation (Boutevin, 1993, Radhakrrishnan, 1994) The UV curing rapidly produces highly cross-linked polymers due to generation of the initiating species at very high rate The highly cross-linked polymers obtained from MFAs or methacrylate monomers produce materials with high structural stability, increased resistance to solvents, and improved mechanical stability These materials are required in diverse applications, such as coatings (of wood, paper and plastics), information storage systems (laser videodiscs and compact discs), stereolithography, spherical lenses, and dental biomaterials (Dietz, 1997) Moreover, the MFAs can also be used as one of the building blocks for interpenetrating polymer networks (IPNs) to improve the properties of commercial materials and tailor new polymers to specific applications

Reactive a-olefinic monomers from vernonia oil

New acrylates and methacrylates were prepared by reacting vernonia oil with an a,b-unsaturated acid (acrylic or methacrylic) In this type of reaction, the carboxylic acid opens the epoxy group of vernonia oil, and a monomer containing three acrylate groups and three hydroxyl groups is obtained The highest conversion of epoxy groups (85-98%) was obtained by performing the reaction with an excess of carboxylic groups at 100-120°C for about 10 h, with and without catalysts Acrylic acid was found to be more reactive than methacrylic acid The structure of the monomers obtained was confirmed by IR and NMR spectroscopy



Vernonia oil triacrylate
R=H,CH_3

The acrylate and methacrylate of vernonia oil were then thermally and photochemically polymerized Thermal cross-linking, which was carried out in the presence of benzoyl peroxide, gave a white amorphous polymer Photochemical cross-linking, which was carried out in the presence of benzophenone, produced transparent colorless films In the IR spectrum of the

polyacrylate, we could distinguish the disappearance of the absorption bands at 1640, 1630, and 810 cm ^{1}, which are characteristic of CH_2=CH, showing that polymerization occured at the terminal double bonds The properties of the polyacrylate and polymethacrylate of vernonia oil—in comparison with those of epoxidized soybean oil—are given in Table 25

Table 25 Mechanical properties of photo-cross-linked polymers

Polymer	Tensile strength, MPa	Elongation,%	Young's modulus, MPa
VO acrylate	2 58	7 63	105 10
VO methacrylate	2 70	11 69	98 85
ESO acrylate	4 76	3 07	252 6
ESO methacrylate	4 31	1 82	482 5

VO - vernonia oil, ESO - epoxidized soybean oil

The tensile properties of the cured systems appeared to be related to the cross-linking density Young's modulus and the breaking strength of the cured films were higher for the acrylate and methacrylate of epoxol (ESO) than for the corresponding acrylate and methacrylate of vernonia oil, because of the higher cross-linking density—and hence the decrease in elongation—of the former

The polymethacrylate of vernonia oil had the lowest value of Young's modulus, a low tensile strength and the highest elongation at break, which made the polymer both soft and tough Dynamic mechanical analysis (DMA) of the polymethacrylate of vernonia oil showed this polymer to be a rubber-like elastomer with a glass transition temperature T_g of -19°C, and therefore that it can be used for the modification of commercially available epoxy resins

Production of an interpenetrating polymer network (IPN)
We used the polymethacrylate of vernonia oil as the elastomer to modify a commercial epoxy resin, Epicote 828 The method of production of a simultaneous interpenetration network (SIN) was used to obtain a combination of two cross-linked polymers the first is a network of the polymethacrylate of vernonia oil and the second is a network of epoxy resin This method involves carrying out two independent, noninterfering reactions simultaneously in the same container under the same conditions Our method for preparing the SIN was based on a condensation polymerization for the epoxy resin and photoinitiated polymerization for the methacrylate of vernonia oil 2, 4, 6-Tris (dimethyl-aminomethyl)phenol (DMP-30) was used as the accelerator for curing the epoxy resin Ultraviolet irradiation polymerization of the methacrylate of vernonia oil was carried out with benzophenone as the photoinitiator Castings were obtained by addition of a mixture of vernonia oil methacrylate with 10 wt % of benzophenone to a mixture of Epicote 828 with 4 wt % of accelerator The formulations were mixed at room temperature to produce a homogeneous mixture The mixture was

degassed with a vacuum pump until bubbling stopped and then poured into the casting Teflon form mold The polymerization was performed in two steps
1 *In situ* UV irradiation polymerization in sunlight for 2 h
2 Heating the formulation at 80°C for 2 h and then at 160°C for 4 h

Casting of the vernonia oil methacrylate was achieved in the first step, and that of the epoxy resin in the second The following combinations of epoxy resin/methacrylate of vernonia oil were prepared 100/0, 84/16, 50/50, 25/75 and 0/100 wt % DMA of the castings showed that all the SIN exhibited only one broad T_g, which was shifted inward from the T_g of cured polymethacrylate of vernonia oil (-19°C) and from the T_g of cured epoxy resin (+105°C) (Table 26) The results obtained showed total compatibility between networks of epoxy resin and the polymethacrylate of vernonia oil

Table 26 Glass transition temperatures for the different IPN formulations

IPN composition epoxy VO methacrylate	T_g, °C
100 0	105
84 16	80
50 50	10
25 75	-4
0 100	-19

The mechanical properties of the castings are shown in Table 27 From Table 27 it can be seen that increasing the amount of soft polymer (the polymethacrylate of vernonia oil) in the SIN sharply decreased both the tensile strength and Young's modulus

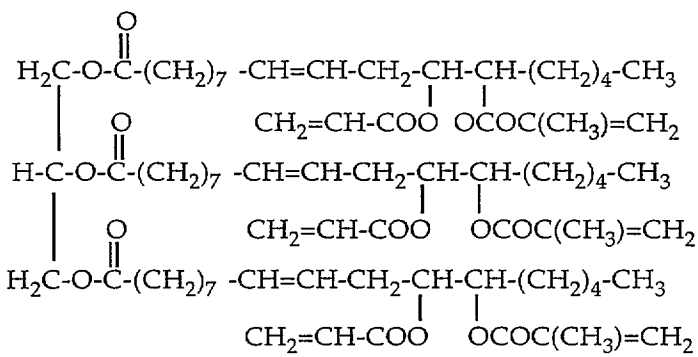
The elongation of the modified epoxy resins was greater (16-18%) than that of the original epoxy resins (5%) The casting containing 50% epoxy resin is a tough elastomer (tensile strength of 16 MPA, and Young's modulus of 322 MPa)

Table 27 Mechanical properties of the castings

Casting composition epoxy resin VO methacrylate, % wt	Tensile strength, MPa	Elongation, %	Young's modulus, MPa
100 0	85	5	2200
50 50	16	16	322
25 75	2	18	20
0 100	1 5	17	13

Acylation of vernonia methacrylate
Additional acrylate bonds were introduced to the methacrylate of vernonia oil in order to make it more active under UV irradiation polymerization These bonds were introduced by acylation of the hydroxyl groups of vernonia oil

methacrylate with acryloyl chloride A complete acylation of the hydroxyl groups of the methacrylate of vernonia oil was obtained by performing the reaction at 0°C for 2-3 hours The structure of the acylated product—the methacrylate monomer of vernonia oil—was confirmed by IR and NMR spectroscopy



Acylated vernonia oil triacrylate

DMA of castings prepared from this new acrylate and Epicote 825 showed that these castings have only one distinct T_g, indicating the compatibility between the networks of the two polymers

We have thus demonstrated the possibility of using the methacrylate and the acrylate-methacrylate of vernonia to prepare mixtures compatible with commercial epoxy resins The results obtained suggest that the combination of these two kinds of polymer (epoxy resins and the polymethacrylate of vernonia oil) will facilitate the preparation of modified epoxy resins with lower brittleness but without deterioration of other properties of the epoxy resins

4 Impact, Relevance and Technology Transfer

This project has enabled a group of scientists in Kenya and Israel to acquire a wide-ranging fund of knowledge about a special new crop, *Vernonia galamensis*, and the unique oil that accumulates in its seeds The members of the group are now very well acquainted with the natural habitats of the plant, its biology, and the variable characteristics of its subspecies and varieties They know how to grow it and what agronomic practices to use, how to hybridize between different germplasms within the species, how to extract the oil, analyze it, and prepare novel products from it using the different functional groups, and how to characterize the unique products (especially polymers) prepared from both the oil and the vernolic acid This knowledge will enable the scientists to carry on with the research, as well as to take on other topics that call for the know-how acquired in the project, such as the development of other oil crops, or of other oils and oil-derived products

To our great and lasting regret, Prof Chyewa passed away before the project ended He was therefore deprived of the opportunity of personally putting to use the experience he himself had gained Hopefully, however, those of his

students who were engaged in this project will be able to utilize their experience

During the project, dozens of new accessions were collected by the team in Kenya and preserved in the Gene Bank of Kenya Since there were only 36 accessions available before initiation of this project, the new accessions represent a very important contribution They are available on request from the Gene Bank Moreover, the Kenyan team has characterized most of the accessions and can recommend the best of them

Given industrial development of its products,*Vernonia galamensis* could become a commercial crop in the near future Since the plant thrives in the tropics, that zone is likely to be the first arena of vernonia exploitation The Kenyan team has gained much experience in growing the plant, both under irrigation and under rain-fed conditions

The Organic Chemistry Laboratory of Prof P Gitu has acquired small- to medium-sized equipment, from hot plate to rotary evaporator, purchased with project funds This equipment strengthens the long-term analytical capacity of that lab, i e beyond the duration of the project

Ms Mary Wawira completed a major part of her work towards an M Sc degree by doing research within the framework of the project in the laboratory of S Grinberg She has successfully completed her degree and is now working on her Ph D thesis

In addition to Ms M Wawira, three other students carried out projects on different topics related to the chemistry of vernonia oil in fulfilment of requirements towards the M Sc degree, under the supervision of Prof P Gitu, Dr B M Bhatt of the Department of Chemistry, University of Nairobi, and Dr S Grinberg of Ben-Gurion University The three projects were (1) "Isolation, Characterization, Chemical Derivatization and Bioassay of Chemical Components of *Vernonia galamensis*", Mr Jeremia K Mwaura, (2) "Chemical Identification and Characterization of Amino and Phosphorylated Derivatives of Vernonia Oil Extracted from *Vernonia galamensis* Seeds", Mr Martin N Mbugua, and (3) " The Synthesis of Vernolamides from *Vernonia galamensis*", Mr Charles M Keyari It should be added that samples of the materials prepared by the students were sent to Israel and analyzed chromatographically and spectroscopically using the facilities at Ben-Gurion University (FT -IR, GC/MS, NMR)

5 Project Activities/Output

5 1 Meetings

In the summer of 1992, S Grinberg attended The Fifth International Chemistry Conference in Africa that was held at Gaborone, Botswana and presented our very first results on the extraction of the oil from the first experimental planting of vernonia in Israel The conference was held just after the project

had been approved for funding At the conference, S Grinberg made the acquaintance of Prof P Gitu, and together they discussed the beginning of the chemistry part of the project

During June 1994, D Mills visited Kenya He joined a short germplasm collection expedition in the dry Kibwezi area with Prof J Chweya and Mr Seme, the head of the Kenyna Gene Bank D Mills visited three of the germplasm trials at Kibwezi and Muguga and helped in the classification and characterization of the plants He also visited the lab of Prof P Gitu and discussed the chemistry part of the project

In November 1994, S Grinberg and D Mills attended the Second European Symposium on Industrial Crops and Products that was held at Pisa, Italy The results concerning the preparation of monoamidoamines and diamines derived from vernonia oil were presented in a paper entitled "New chemical derivatives based on *Vernonia galemensis* oil"

Prof P Gitu arrived together with Ms Wawira for a one month's visit (August 1994) in the lab of S Grinberg, discussed different aspects of the research project, and visited organic chemistry labs at Ben-Gurion University, at the Hebrew University of Jerusalem and at the Weizmann Institute of Science, Rehovot

In February 1996, S Grinberg attended The 61st Meeting of the Israel Chemical Society, which was held in Jerusalem, Israel, and presented the work entitled "New polymers from triglyceride oils containing epoxy groups "

In April 1996, D Mills and S Grinberg attended the Third European Symposium on Industrial Crops and Products held at Reims, France and presented the work entitled "Hybridization trials and seed oil composition of *Vernonia galamensis*"

On 8-10 of July, 1996, Prof Chweya attended the "International Symposium on Domestication, Production and Utilization of New Crops Practical Approaches", held at the University of Southampton and organized by the International Centre for Underutilized Crops The title of the abstract submitted was "Characterization and Evaluation of some *Vernonia galamensis* Accessions Collected in Kenya" by J A Chweya, E N Seme and P A Amuyunzu The abstract appears in a book entitled "Domestication, Production and Utilization of New Crops" edited by J Smart and N Haq, Overseas Development Administration (ODA), UK 1997 Page 293

D Mills attended the Conference of the American Association for the Advancement of Industrial Crops held in Saltillo, Mexico, in October 1997 He presented his work entitled "Germplasm improvement in *Vernonia galamensis*" S Grinberg submitted an abstract entitled"*Vernonia galemensis* A renewable resource for reactive monomers and their corresponding polymers"

In October 1998 D Mills attended the Conference of American Association for the Advancement of Industrial Crops held in Phoenix, Arizona He presented

his work entitled "Effect of liquid fertilizer on Vernonia Galamensis grown in the greenhouse"

Lastly, D Mills attended the Conference of American Association for the Advancement of Industrial Crops held in Eugene, Oregon, in October 1999 He presented his work entitled "Hybridization trials in Vernonia Galamensis"

5 2 Training

Ms Mary Wawira, a student in the Department of Chemistry at the University of Nairobi, spent about a year (August 1994-August 1995) in the chemistry laboratory at the Institutes for Applied Research of Ben-Gurion University of the Negev, where she carried out the bulk of her work on an M Sc thesis entitled "Extraction and characterization of vernonia oil and synthesis of N-(-aminoalkyl)vernolamides and N,N'- alkylene bis(vernolamide)" She studied the reaction of vernonia oil with 1,4-diaminobutane as a model reaction Ms Warnira investigated the effect of different parameters such as molar ratio oil diamine, temperature, and solvent on the course of the reaction She used TLC, GC and HPLC analysis to follow product formation in the reaction The products were separated and spectroscopically characterized (IR, NMR)

Ms Wawira acquired expertise in the use of new analytical procedures and worked with instrumentation that is rarely available in the labs in Kenya

5 3 Publications

Grinberg, S , Y Kolot, and D Mills 1994 New chemical derivatives based on *Vernonia galamensis oil* Industrial Crops and Products 3 113-119

Mills D Response of *Vernonia galamensis* grown in the greenhouse to liquid fertilization J of Plant Nutrition 22 1665-1678

Five manuscripts are being prepared for publication by D Mills and S Grinberg

6 Project Productivity

Thanks in part to the fact that the duration of the project was extended to over five years, we have met all of the initial objectives, some with greater success and others with less

The germplasm collection effort resulted in the addition of 53 new accessions to the quite limited collection of 36 accessions available worldwide (Perdue et al 1989 a, 1989b) These accessions were evaluated in three locations for selection of superior specimens Commercial trials were then carried out together with studies of agromanagement elements such as topping and plant density Due to unexpected factors, including shortage of rain, yields in the commercial trials were lower than in earlier trials, causing some uncertainty regarding previous estimates of potential yields of the selected accessions An intraspecific

hybridization study followed five generations of recurrent selections, two more than initially planned The particular selection path adopted resulted in two lines that flower in summer, have a medium-to-high self-compatibility, but contrary to earlier expectations do have not many flowerheads The potential of these lines has yet to be elucidated

The chemistry of vernonia oil and vernolic acid was thoroughly and extensively studied New chemical derivatives and new polymers based on vernonia oil as a renewable resource were prepared by exploiting the chemical multi-functionality of the oil Reactions were performed on all the functional groups Brominated compounds were obtained by performing addition and allylic bromination on the double bonds of the methyl vernolate and the oil itself

Aminolysis of vernonia oil was performed by reacting the oil with aliphatic diamines, followed by isolation and characterization of the intermediate—the monoamidoamine of vernolic acid—and the final product—N,N'-alkylbis(vernolamide) The fatty bisamides two epoxy groups in the molecule and represent new reactive difunctional epoxy monomers By reacting vernonia oil with aromatic diamines, a series of new rubber-like polymers were obtained The gel fraction content of the cured polymers ranges between 45% and 70%, and as expected they are more thermostable than the original vernonia oil These polymers were then used to modify commercial epoxy resin (Epicote 828) in order to obtain interpenetrating networks (IPNs) The formulation were prepared by adding prepolymers of vernonia oil with *m*-phenylene diamine (or epoxol with m-*m*-phenylene diamine) to a mixture of Epicote 828 in the presence of an accelerator These formulations were mixed at room temperature to produce homogeneous transparent mixtures The transparency of the mixtures obtained is evidence of the compatibility between epoxy resin (Epicote 828) and the corresponding prepolymer The IPN containing 50% vernonia oil polyamine, for example, is a reinforced elastomer with an elongation that is three times greater than that of the polyamine of vernonia oil

Acrylation and methacrylation of vernonia oil gave new reactive a-olefinic monomers These multifunctional acrylates (MFAs) were photochemically cross-linked, in the presence of benzophenone, to produce transparent colorless films The polymethacrylate of vernonia oil was used as elastomer to modify a commercial epoxy resin, Epicote 828, to obtain interpenetrating networks (IPNs) Casting of the vernonia oil methacrylate was achieved in the first step and casting of the epoxy resin in the second DMA of the castings showed that all the SIN exhibited only one broad T_g, which was shifted inward from the T_g of the cured polymethacrylate of vernonia oil (-19°C) and from the T_g of the cured epoxy resin (+105°C) The results obtained showed total compatibility between networks of epoxy resin and the polymethacrylate of vernonia oil The casting containing 50% epoxy resin is a tough elastomer (tensile strength of 16 MPA, and Young's modulus of 322 MPa)

In the future, vernonia oil will compete with partially or fully epoxidized soybean oil (ESBO) Consequently, parallel to exploring the preparation of

novel products from vernonia oil, it will be necessary to examine similar preparations from ESBO and identify those products that would benefit from the use of vernonia oil rather than ESBO

7 Future Work

D Mills will continue screening inbred offspring with the aim of achieving higher flowerhead production and higher seed yields He is planning to cross the F5 hybrids with var *petitiana* and possibly with var *galamensis* (backcross) He will also examine performance of a certain accession obtained from Sedamo area in Ethiopia This is a *Vernonia galamensis* var *petitiana* that was collected north-west of Yabello and is native to arid zones It has a high self-compatibility

S Grinberg will continue to use vernonia oil or derivatives based on it as reactive epoxy monomers to obtain the corresponding polymers The latter will then be taken as elastomeric component in the preparation of new IPNs Fatty bisamides, which contain two epoxy groups in the molecule, are new reactive difunctional epoxy monomers The bis(alkylenevernolamides) will be taken as starting material to synthesize new reactive acrylic monomers The mechanical properties of the films obtained from the acrylated amides will be compared with those of the films obtained on the base of acrylated vernonia oil, in order to find proper applications for each one of the polymers

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