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

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EXECUTIVE SUMMARY

The report is divided to two parts, the first deals with the biological/agronomical work and the second with the chemistry work

In the biological section of the program, we continued with selection of lines of the hybrids prepared at the beginning of this project This year, we tested the F3 lines of between var *galamensis* and var *ethiopica* and the F2 backcross of the hybrid between ssp *galamensis* var *petitiana* (accession V029) and var *ethiopica* Tests were conducted in the greenhouse in winter and in the field during summer As in previous years, hybrids exhibited characteristics intermediate between the parent varieties, and in some cases even similar characteristics as those of the parent, such as seed filling and seed weight as in var *ethiopica* Some lines may be considered good as they combine positive characteristics of both parents, referring mainly to neutral flowering (trait of var *galamensis*) with big seeds and capitulum and high seed filling (traits of var *ethiopica*) The F2 backcross lines of the hybrid between var *petitiana* and var *ethiopica* also exhibited intermediate characteristics, as reflected by mainly the size of the capitula and the number of capitula These hybrid lines exhibited similar performance and characteristics to the hybrid lines of var *galamensis* and var *petitiana* but had the inferiority of having small capitula and seeds It was decided to continue selection within these lines Of the six lines tested, we selected F4 seeds presenting excellent features high seed filling, big seeds, numerous and big capitula, and early flowering These traits are, however, not within one line, meaning that further selection has to be made We envisage that by performing further selection we can come up with a line characterized by early flowering in summer (neutral to day length) and reasonable yields

In Kenya, further field testing—aimed at commercialization—was conducted The trials were conducted under dry-land farming conditions Low yields were obtained in the two sites, Kabete and Kibwezi, due to a dry spell during the period of growth and to interference by wild animals Var *ethiopica* was more susceptible to the drought than the other subspecies tested In both locations, subspecies *mutomoensis* yielded more than the other germplasm

In the chemistry part of the project, chemical transformations of vernonia oil, vernolic acids and methyl vernolate, with the aim of preparing surfactants, were performed under basic conditions Transmethylation and amidation did not result in epoxy ring opening Partial opening of the epoxy ring was associated with the preparation of sodium and potassium vernolic salts from vernolic methyl esters or acids by the action of sodium hydroxide, potassium hydroxide or sodium carbonate

The properties (solubility in water, critical micelle concentration and foaming properties) of the new compounds indicate that vernolic salts and diethanolamides are good candidates for detergents and thickening agents i e , in the common fields of application of analogous surfactants

The formation of estolides as by-product is of prime interest since this can offer simple method of producing new types of estolides These estolides can form the basis of lubricants with unique properties

GENETIC IMPROVEMENT OF VERNONIA AND COMMERCIAL TRIALS

INTRODUCTION

Vernonia galamensis is a new oil seed plant It is distributed widely throughout Africa—from areas with an annual rainfall as low as 200 mm (Perdue 1989) to rich forested areas with an annual rainfall of 1,850 mm The species *galamensis* is made up of six subspecies The most important germplasm of *V galamensis* is accession V-001 (subsp *galamensis* var *ethiopica*), which was collected by Dr Perdue near Harer, Ethiopia in 1964 (Perdue et al 1989) Plants of this accession are high yielding and produce big seeds that are firmly attached in the flower head when ripe The variety is, however, sensitive to cold, and flowering occurs in response to short day length, a characteristic that is highly undesirable in a crop grown in cold-prone areas outside tropical zones Other accessions such as V-004 and V-029 have been reported to flower in conditions of long day length (Phatak 1989) This has been confirmed by observations made in Israel (Mills 1991) and in the US (Thompson et al 1994)

The seeds of the plant contain high levels of an oil rich in epoxy acids (Perdue et al 1989) Since epoxy oils (mainly epoxidized soybean oil) are used in various chemical industries for the production of plastics, protective coatings, and other organic formulations, efforts are being made to develop this plant as a new industrial crop The seed of *V galamensis* contains about 40% oil, of which the vernolic acid (*cis*-12,13 epoxy, cis-9-octadecenoic acid) content is 72-80% Vernolic acid is primarily present in the oil as a triglyceride, which is known as trivernolin

In Israel, in the biology part of the program, we demonstrated—in terms of phenological characteristics—that hybridization between varieties of

ssp *galamensis* is possible (Mills et al 1995) Most promising crosses are those of var *ethiopica* (accession V-001) either with var *petitiana* (accession V-029) or var *galamensis* (accession V-004) F1 hybrids (first generation progenies) or backcrosses with V001 flowered during the summer Their yield potential has yet to be evaluated The most important finding was the fact that hybrid plants flowered in the summer under conditions of long days, i e , conditions under which the V-001 parent does not flower Hybrid plants flowered after a slightly longer period than that of the other parent, V004 (45-53 days) The backcross of the hybrid between V029 and V001 with *ethiopica* (V001), which is similar to *ethiopica* in phenological characteristics, flowered after three months Here we report on the performance of the F3 hybrid plants and the backcross plants The evaluation was carried out in the greenhouse and in the field, and a comparison between the two sites is presented

In the biology part of the program in Kenya, the first evaluation of the germplasm collected was performed Evaluation took place at the Kibwezi Dry Land Field Station (representing marginal lands) and the Kabete Field Station of the University of Nairobi (representing high-potential areas) Significant differences in phenological characteristics and yield were found among 43 accessions of five subspecies 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 effect of plant density on yield was found The two experiments on topping and plant density will be repeated in the next season The yields (2-4 tons seeds per hectare) reported by the Kenyans, by extrapolating from the small plots, are very encouraging and are higher than the few figures reported in the literature so far These yield data must, however, be verified in a large-scale field trial Recently, the growing of vernonia as a commercial crop has been attempted Three accessions representing three subspecies were planted at the Kabete Field Station, while another five representing five subspecies and varieties were planted at Kibwezi

RESULTS AND DISCUSSION

1 CHARACTERIZATION OF HYBRID PLANTS BETWEEN VAR *ETHIOPICA* AND VAR *PETITIANA* OR *GALAMENSIS* (ISRAEL)

1 1 F3 hybrids & backcrosses (greenhouse 1996/7)

Seeds of the selected F3 hybrids and the backcross V029 × V001 BC F2 were sown in the greenhouse at the end of September 1996 Dates of flowering were recorded, and other characteristics were measured during growth and after harvest (in July 1997) when most plants had matured and dried-up

In general, F3 hybrids between var *galamensis* and var *ethiopica* showed phenological characteristics that are intermediate between those of the parents, (Table 1) Var *galamensis* was not grown in this season, 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 (V001 × V004) were closer to it than when it served as the male parent (V004 × V001) (Table 1) This is reflected in the following characteristics height of the plant, leaf length/width ratio and receptacle diameter The situation was different for the F2 hybrids When var *ethiopica* served as the male parent, the F2 hybrids were closer to it than when it served as the female parent (Mills et al 1997) In addition, in contrast to F2 hybrids, which flowered significantly earlier than var *ethiopica*, F3 hybrid plants flowered only slightly earlier (with referrence to the V001 data of last year)

Three F3 lines of each type of hybridization, either V001 × V004 or V004 × V001, were tested Great variability was found within each group (Table 3) In the group of V004 × V001, line D3h excelled in the number of flowers per plant and in the seed weight, line B7a/gh excelled in the width of the capitulum and early flowering, and line F6h 2/3f in seed filling The latter line was generally inferior to the other two In the group of V001 × V004, line f3/3 excelled in flower production, line f3/2 in seed biomass and width of capitulum, and line A6C/gh in seed filling In general, lines of group V001 × V004 performed worse than lines of the group V004 × V001

It should be emphasized that in relation to var *ethiopica* (V001), the hybrid plants exhibit superiority If we take line D3h, for example, it is

characterized by similar seed filling and seed weight to V001, but it has many more capitula and flowers earlier than V001

The F2 backcross of the hybrid between ssp *galamensis* var *petitiana* (accession V029) and var *ethiopica* as the male parent (V001) exhibited intermediate characteristics, as reflected mainly in the size of the flower head and the number of flowers (Table 1) These hybrids exhibited similar performance and characteristics to the hybrids of var *galamensis*, but were inferior in terms of capitulum size and seeds There were some large differences between the two lines C2b and C1a with reference to flower and seed production (Table 3) Line C1a had numerous capitula, many more than line C2b, but it produced no seeds

Selection of new high potential offspring from F3 hybrids, V004 × V001 (D, F6h 2/3f, B7a/gh,D), V001 × V004 (f2/3, f3/3, A6C/gh) and V029 × V001BC (C2b) was performed on the basis of the performance of individual plants of the three hybrids (Tables 4-10, respectively) Important characteristics for selection of superior plants were 1) early flowering, 2) numerous flower heads, and 3) high percentage of full seeds Large receptacles and heavier seeds were considered less important Accordingly, six F4 lines were chosen for continuing the selection in the winter of 1997/8

1 - plant no 4 of hybrid D3h (V004 × V001) (Table 5) that excelled in high seed filling, early flowering and large capitula
2 - plant 12 of F6h(f2/3) (V001 × V004) (Table 6) that excelled in high seed filling, numerous and large capitula
3 - plant 13 of hybrid B7a/gh (V004 × V001) (Table 7) that excelled in high seed filling, early flowering and numerous capitula
4 - plant 1 of hyb f3/2 (V001 × V004) (Table 8) that excelled in high seed filling and weight and early flowering
5 - plant 5 of hyb f3/2 (V001 × V004) (Table 8) that excelled in high seed filling, early flowering and large capitula
6 - plant 12 of hyb f3/2 (V001 × V004) (Table 8) that excelled in numerous large capitula

Table 1 Phenological characteristics of ssp *galamensis*, var *ethiopica* (accession V001) and hybrids with ssp *galamensis*, var *galamensis* (V004) grown in the greenhouse in 1996/7
Each value is a mean of 11-36 plants of the group
Five branches, flowers and leaves were measured in each plant

Phenological parameter	V001	V004 x V001 F3	V001xV004 F3	V029xV001 BC F2
Plant height (cm)	83 b	78 4 b	80 8 b	56 4 a
Height of first flower (cm)	35 0 c	29 5 bc	26 1 ab	21 8 a
Number of primary branches	5 8 a	7 2 b	7 6 b	7 2 ab
Leaf width (cm)	1 28 ab	1 57 c	1 35 b	1 12 a
Leaf length (cm)	11 9 c	11 9 c	11 0 b	8 8 a
Leaf length/width	9 62 c	7 74 a	8 39 b	7 60 a
Receptacle diameter (mm)	1 87 d	1 09 b	1 33 c	0 88 a
Involucre length (mm)	1 84 b	1 50 a	1 50 a	1 49 a
Ratio of full seeds (% of total)	44 5 b	43 4 ab	32 6 a	36 1 ab
One thousand seed wt (g)	6 766 c	5 800 b	5 312 b	4 381 a
Number of capitula	47 a	240 b	198 b	221 b
Flowering (days from sowing)	104 b	95 a	95 a	96 ab

Table 2 Phenological characteristics of ssp *galamensis* var *galamensis* (accession V004) and var *ethiopica* (accession V001) and their F3 hybrids, and of the backcross of the hybrid between ssp *galamensis* var *petitiana* (accession V029) and var *ethiopica* as the male parent (V001) grown in the greenhouse in 1996/7
Each value is a mean of 5-105 plants of the group
Five branches, flowers and leaves were measured in each plant

Phenological parameter	V001	V004 x V001 F3			V001 x V004 F3		
		D3h	B7a/gh	F6h 2/3f	f3/3	f3/2	A6C/gh
Plant height	83 cde	70 bc	90 de	73 bc	74 bcd	92 e	66 ab
No of branches below 1st flower	5 8 a	6 3 ab	7 6 bc	7 7 bc	10 1 d	7 2 abc	7 2 abc
Leaf width (cm)	1 3 a	1 4 a	1 6 b	1 7 c	1 5 b	1 3 a	1 3 a
Leaf length (cm)	11 9 b	9 1 a	12 7 c	13 0 c	12 3 bc	11 8 b	9 1 a
Leaf length/width	9 62 c	6 81 a	8 27 b	7 81 ab	8 39 b	9 11 c	7 22 a
Receptacle diameter (mm)	1 87 e	0 94 a	1 23 c	1 06 b	1 19 c	1 46 d	1 17 c
Involucre length (mm)	1 85 e	1 51 bcd	1 53 cd	1 47 ab	1 56 d	1 53 cd	1 43 a
Number of capitula	48 a	311 d	259 cd	165 bc	255 bcd	194 bc	180 bc
Ratio of full seeds (% of total)	44 5 b	35 9 ab	27 2 a	44 4 b	37 3 ab	28 1 a	40 8 ab
One thousand seed wt (g)	6 766 d	6 287 cd	5 230 abc	6 063 cd	5 077 ab	5 534 bc	4 898 a
Flowering (days from sowing)	104 cd	92 b	83 a	109 d	97 bc	96 b	92 b

Table 3 Phenological characteristics of the backcross of the hybrid between ssp *galamensis* var *petitiana* (accession V029) and var *ethiopica* as the male parent (V001) grown in the greenhouse in 1996/7
Each value is a mean of 5-70 plants of the group
Five branches, flowers and leaves were measured in each plant

Phenological parameter	V001	V029 X V001 C2b	BC F2 C1a
Plant height	83 b	53 a	85 b
Height of first flower (cm)	35b	20a	n d
No of branches below 1st flower	5 8 a	6 9 ab	9 5 b
Leaf width (cm)	1 3 b	1 1 a	1 6 c
Leaf length (cm)	11 9 b	8 7 a	10 a
Leaf length/width	9 62 b	7 69 a	6 33 a
Receptacle diameter (mm)	1 87 b	0 89 a	0 78 a
Involucre length (mm)	1 85 b	1 49 a	1 52 a
Number of capitula	48 a	157 b	1123 c
Ratio of full seeds (% of total)	44 a	43 a	no seeds
One thousand seed wt (g)	6 766	4 381	no seeds
Flowering (days from sowing)	104 b	95 a	97 ab

Table 4 Phenological characteristics of individual plants of the F1 backcross of the hybrid between ssp *galamensis* var *petitiana* (accession V029) and var *ethiopica* with the male parent (V001), designated V029 × V001 BC 2(C2b), grown in the greenhouse in 1996/7
Superior measurements indicated in bold

Plant no	Receptacle diameter (mm)	Number of capitula	Flowering (days from sowing)	Full seeds (% of total)	One thousand seed wt (g)
1	76	73	91	25 0	4 334
2	92	240	97	51 5	**5 067**
3	**1 1**	183		**77 0**	4 46
4	**1 08**	51	**84**	**71 4**	3 622
5	76	55	97	35 5	**5 071**
6	96	**408**	91	-	-
7	72	52	-	-	-
8	*	-	104	-	-
9	-	-	**84**	-	
10	9	**415**	96	-	-
11	88	135	97	-	-
12	84	82	91	31 5	**5 153**
13	82	216	103	44 4	3 239
14	**1 06**	72	100	26 3	4 579
15	88	152	104	30 1	4 464
16	74	60	100	41 8	3 822

*- = not determined

Table 5 Phenological characteristics of individual plants of the F3 hybrids between ssp *galamensis* var *galamensis* (accession V004) and var *ethiopica* (accession V001), designated V004 × V001 F3 (D3h), grown in the greenhouse in 1996/7
Superior measurements are indicated in bold type

Plant no	Receptacle diameter (mm)	Number of capitula	Flowering (days from sowing)	Full seeds (% of total)	One thousand seed wt (g)
1	*	-	**83**	-	-
2	66	103	-	-	-
3	78	**621**	103	-	-
4	**1 32**	224	**83**	**71 4**	5 947
5	-	-	97	-	-
6	68	157		-	-
7	76	302	111	10 4	**7 07**
8	66	160		34 1	6 064
9	88	63	**83**	27 5	6 067
10	1 22	**681**	106	-	-
11	-	-	**83**	-	-
12	1 18	101	**83**	-	-
13	-	-	**83**	-	-
14	**1 24**	**695**	100	-	-

*- = not determined

Table 6 Phenological characteristics of individual plants of the F3 hybrids between ssp *galamensis* var *galamensis* (accession V004) and var *ethiopica* (accession V001), designated V004 ×V001 F3 (F6h f2/3), grown in the greenhouse in 1996/7
Superior measurements are indicated in bold type

Plant no	Receptacle diameter (mm)	Number of capitula	Flowering (days from sowing)	Full seeds (% of total)	One thousand seed wt (g)
1	1 02	187	**95**	18 3	5 73
2	1 12	199	119	46 6	4 981
3	72	65	121	36 5	**7 233**
4	**1 2**	145	**99**	36 3	5 768
5	98	89	104	56 5	5 43
6	92	**290**	**100**	**68 9**	4 934
7	1 04	128	107	31 9	**8 043**
8	1 12	**331**	115	51 2	6 667
9	84	35	11	32 8	5 566
10	**1 4**	140	120	*-	-
11	1 16	174	**100**	40 5	6 791
12	**1 32**	**250**	107	**74 1**	6 325
13	98	118	121	38 9	5 285

*- = not determined

Table 7 Phenological characteristics of individual plants of the F3 hybrids between ssp *galamensis* var *galamensis* (accession V004) and var *ethiopica* (accession V001), designated V004 × V001 (B7a/gh), grown in the greenhouse in 1996/7
Superior measurements are indicated in bold type

Plant no	Receptacle diameter (mm)	Number of capitula	Flowering (days from sowing)	Full seeds (% of total)	One thousand seed wt (g)
1	**1 3**	2 03	**83**	1 6	-
2	**1 52**	76	78	32 4	**6 087**
3	*-	-	85		-
4	**1 34**	**404**	**83**	1 4	-
5	1 26	98	96	-	-
6	**1 34**	125	101	32 0	4 027
7	1 1	159	**79**	23 1	5 527
8	**1 32**	**404**	-	23 1	**6 084**
9	1 2	170	**79**	-	-
10	1 16	**355**	-	28 6	**6 164**
11	1 16	262	80	**56 3**	4 447
12	1 22	371	**78**	-	-
13	1 14	**359**	**74**	**46 5**	4 418
14	98	283	-	32 3	5 277
15	1 16	**358**	85	22 2	5 04

*- = not determined

Table 8 Phenological characteristics of individual plants of the F3 hybrids between ssp *galamensis* var *ethiopica* (accession V001) and ssp *galamensis* var *galamensis* (accession V004), designated V001 × V004 F3 (f3/2), grown in the greenhouse in 1996/7
Superior measurements are indicated in bold type

Plant no	Receptacle diameter (mm)	Number of capitula	Flowering (days from sowing)	Full seeds (% of total)	One thousand seed wt (g)
1	1 52	178	**86**	**50 5**	**6 081**
2	**1 88**	217	100	30 1	5 548
3	1 3	108	**86**	16 6	4 400
4	1 4	164	105	30 9	5 078
5	**1 98**	**399**	97	**46 5**	5 591
6	1 36	155	**91**	24 1	4 976
7	**1 78**	195	*-	38 2	7 1
8	1 42	255	100	**45 4**	5 55
9	1 34	116	96	18 1	6 955
10	1 46	188	**91**	**44 1**	5 852
11	1 48	137	99	18 8	4 607
12	**1 7**	**402**	-	43 5	5 315
13	1 52	195	-	32 7	5 833
14	1 4	261	106	19 8	4 615
15	1 2	183	97	21 1	4 329
16	1 16	263	100	28	5 295
17	1 26	122	-	18 2	**6 144**
18	1 34	118	97	16 0	4 309
19	1 24	183	98	30 3	5 547
20	1 6	139	97	16 0	**7 392**
21	1 32	94	**91**	1 8	5 700

*- = not determined

Table 9 Phenological characteristics of individual plants of the F3 hybrids between ssp *galamensis* var *ethiopica* (accession V001) and ssp *galamensis* var *galamensis* (accession V004), designated V001 ×V004 F3 (f3/3), grown in the greenhouse in 1996/7
Superior measurements are indicated in bold type

Plant no	Receptacle diameter (mm)	Number of capitula	Flowering (days from sowing)	Full seeds (% of total)	One thousand seed wt (g)
1	1 1	161	105	38 9	4 229
2	1 24	181	*-	**50 0**	3 42
3	**1 56**	220	**91**	**45 5**	**6 786**
4	1 32	-	**87**	-	-
5	1 13	94	**91**	14 7	5 875
6	74	**619**	109	-	-

*- = not determined

Table 10 Phenological characteristics of individual plants of the F3 hybrids between ssp *galamensis* var *ethiopica* (accession V001) and ssp *galamensis* var *galamensis* (accession V004), designated V001 × V004 F3 (A6c/gh), grown in the greenhouse in 1996/7
Superior measurements are indicated in bold type

Plant no	Receptacle diameter (mm)	Number of capitula	Flowering (days from sowing)	Full seeds (% of total)	One thousand seed wt (g)
1	1 16	137	**80**	41 7	4 988
2	*-		96	-	-
3	-	-	**79**	-	-
4	**1 38**	224	-	**50 3**	4 216
5	1 16	**246**	-	14 6	**6 009**
6	1 02	148	102	41 7	5 108
7	1 28	147	-	39 4	5 422
8	**1 5**	166	-	**61 2**	4 34
9	1 16	202	-	49 5	**6 022**
10	1 0	96	100	38 5	3 722
11	1 02	123		30 3	4 255
12	1 07	-	**83**	-	-
13	-	-	100		-
14	**1 34**	**253**	97	-	
15	86	**235**	-	-	

*- = not determined

1 2 F3 hybrid (field 1997)

Seeds collected from bagged flowers of F2 hybrids grown in the field in 1996 or in the greenhouse in 1995/6 were sown in the greenhouse and transplanted into the field in April 1997 The F3 V004 ×V001 and V001 × V004 hybrids flowered (50% flowering) 81 and 96 days after sowing, respectively (Table 11), var *galamensis* (V004) flowered 85 days after sowing, and var *ethiopica* had not flowered by the time final records were made Nevertheless, plants of var *ethiopica* (V001) were left in the field (with irrigation), and flowering was observed at the end of October It should be mentioned that these plants (V001), along with other lines that had been left in the field, suffered significantly during the cold days of winter, again supporting the premise that vernonia cannot be cultivated under the conditions of the northern Negev The ratio of leaf length to leaf width for V001 × V004 F3 hybrids was similar to that for var *ethiopica*, while F3 plants of the reciprocal cross (V004×V001) had leaves of intermediate size compared to the parents (Table 11), as was observed with F1 and F2 hybrids (Mills et al 1995 and 1997, respectively) Intermediate characteristics to the parents were also found for the height, the number of capitula and the dimensions of the flower head (involucre length and receptacle diameter), i e , F3 plants are intermediate to the parents The shape of the capitulum of V004×V001F3 hybrids was short and wide, while that of V001×V004 was longer and narrower

In general, growth patterns and parameters of F2 hybrids and mother plants grown in the field in 1996 were similar to those of the F1 hybrids and mother plants in 1995 For example, the time to flowering of the hybrids was close to the that of var *galamensis* In both years—1995 and 1996—the hybrid between var *galamensis* and var *ethiopica* (V004×V001 F1 and F2) flowered earlier than the reciprocal cross (V001 × V004 F1 and F2) However, some differences were noted In the F2 hybrids, the flower head dimensions were similar to those of F1 hybrids grown in 1995, except for receptacle diameter of V001×V004, which was significantly smaller (by 2 5 mm) This difference is inherited, since it also appears in F3 plants of the reciprocal cross, which had a smaller receptacle diameter (Table 11)

Table 11 Phenological characteristics of ssp *galamensis* varieties *ethiopica* (V-001), *galamensis* (V-004) and *petitiana* (V-029) and F3 hybrids of ssp *galamensis* var *galamensis* (accession V004) with ssp *galamensis* var *ethiopica* (V001) four months after transplanting in the field in April 1997 The two hybrid populations consisted of 3 hybrid lines each (see Table 12) Five branches, flower heads and leaves were measured in each plant Statistically significant differences (P<0 05) between means are designated by different letters

Phenological parameter	V-001	V 004	V 029	V004 x V001	V001 x V004
Plant height (cm)	96 b	83 b	73 ab	82 b	58 a
Height of first flower (cm)	96 b	30 a	32 a	34 a	28 a
Leaf width (cm)	1 24 c	1 10 b	1 02 b	1 18 c	0 89 a
Leaf length (cm)	14 6 d	9 7 b	8 4 a	11 25 c	9 84 b
Leaf length/width	12 0 c	8 9 a	8 4 a	9 8 b	11 4 c
Receptacle diameter (mm)	1 72 d	0 77 a	0 73 a	1 23 c	1 08 b
Involucre length (mm)	1 55 e	1 11 b	0 96 a	1 36 d	1 29 c
Flowering level*	2 3 a	4 9 b	4 7 b	3 0 a	3 1 a
Number of capitula	42 a	282 c	179 b	63 a	47 a
Flowering (days from sowing)**	***	86	79	81	96

* Number of levels (primary flower = level 1, secondary flowers = level 2, etc),
** When 50% of plants flowered, ***flowered at end of October

Table 12 Phenological characteristics of F3 hybrids of ssp *galamensis* var galamensis (accession V004) with ssp *galamensis* var *ethiopica* (V001) four months after transplanting in the field in April 1997 Each plant population (e g , B7a/gh) originated from a single F2 hybrid plant
Each value is a mean of 10-30 plants of the group (in 3 blocks) Five branches, flower heads and leaves were measured in each plant Statistically significant differences (P<0 05) between means are designated by different letters

	V004 xV001 F3			V001 xV004 F3		
Phenological parameter	D f5/7	B7a/gh	F6h 2/3f	f3/3	f3/2	A6C/gh
Plant height (cm)	100 d	66 ab	72 bc	54 a	80 bc	54 a
Height of first flower (cm)	51 b	22 a	16 a	32 ab	40 ab	8 a
Leaf width (cm)	1 30 f	1 12 e	0 98 bc	0 84 a	0 87 ab	1 1 cde
Leaf length (cm)	10 8 d	11 7 e	11 2de	10 2 cd	8 7 a	9 3 abc
Leaf length/width	8 5 a	10 6 c	11 4 cd	12 3 d	11 4 cd	8 7 ab
Receptacle diameter (mm)	1 26 bc	1 15 b	1 25 bc	1 0 a	1 3 c	1 21 bc
Involucre length (mm)	1 33 ab	1 43 b	1 28 ab	1 19 a	1 57 c	1 46 bc
Flowering level*	2 7 a	3 0 a	4 0 b	3 0 a	3 3 a	3 3 a
Number of capitula	89 a	42 a	42 a	45 a	57 a	43 a
Flowering (days from sowing)**	92	76	77	104	107	60

* Number of levels (primary flower = level 1, secondary flowers = level 2, etc)
** When 50% of plants flowered

Analyzing the differences between the three lines of each type of cross, we find that D f5/7 is different from the other two lines of the cross of V004 × V001 It is a taller plant (has longer branches) with more capitula, but it flowers late In the reciprocal cross (V001× V004), line A6C/gh flowered significantly earlier than the other lines and had wider leaves (Table 12) The line f 3/3 had smaller capitula than the other two lines

1 3 Comparison of characteristics of plants grown in the field and the greenhouse

Since the same hybrids were grown in the field and the greenhouse, it is possible to compare performance and phenological characteristics of the two groups of plants Conditions of growth in the greenhouse are much closer to optimal conditions than growth in the field Temperature is controlled and irrigation and fertilization are better regulated Pests and weeds are much less frequent in the greenhouse and are also better controlled The pattern of growth in the greenhouse was different to that in the field, e g , in the greenhouse, leaves were significantly broader and shorter, and differences in capitulum dimensions of some lines were observed Plants were much more productive in the greenhouse We reported such differences in our last annual report (Mills et al 1997) and pointed out that it is difficult to analyze phenological characteristics by combining the results for the greenhouse and the field, because differences in performance in the two types of site We should mention, though, that this year's data showed less variation in performance between the two sites

1 4 Selection of F3 hybrid lines for continuing selection

F3 plants exhibited significant variability in the field in all phenological characteristics dimensions of the canopy, numbers of branches and extent of branching, capitula production, and dimensions of leaves and of capitula We felt that it was difficult to base selection on field performance and rather based it on performance in the greenhouse, which is more reliable, since growth takes place under more uniform conditions of fertigation and pest and disease control We still wanted to continue with most of our lines, so individual plants were selected from six lines (see above) We omitted the back cross between var *petitiana* and var *ethiopica* (V029 x V001 BC F2) mainly due to the small size of the capitulum and seed and due to low seed filling (all contributing to lower yields)

The selected F4 seeds have excellent features high seed filling, big seeds, numerous and large capitula, and early flowering These traits, however, are not within one line, meaning that further selection has to be made We envisage that by carrying out further selection we can come up with a line characterized by early flowering in summer (neutral to day length) and reasonable yields

2 FIELD WORK IN KENYA

2 1 Commercial trials for growing vernonia

Commercial trials for growing vernonia were carried out both at the Kabete Field Station and the Kibwezi Dry Land Field Station of the University of Nairobi under conditions of dry land farming

The experiment at Kabete was characterized by a dry spell during most of the growing period Therefore, the difference in the ability of plants to tolerate or withstand this dry spell was evident Var *ethiopica* was particularly affected by the drought, more so than the other two subspecies (*mutomoensis* and *gibbosa*), which showed average performance The results on days to 50% flowering, harvesting period and seed and oil yields are presented in Table 13 Subspecies *mutomoensis* gave the best yield (475 kg/ha), followed by subspecies *gibbosa* (378 kg/ha) and lastly var *ethiopica* (351 kg/ha) However, var *ethiopica* had the shortest harvesting period (104 days) as compared to the other two subspecies, which had a harvesting period of 118 days

The results for the experiment in Kibwezi are presented in the Table 14 Seed yield from this experiment was, however, lower than that obtained from the experiment at Kabete (Table 13) due to some external interference from wild animals, resulting in destruction of some of the plants However, like the experiment in Kabete, there was a similar pattern in the amounts of seed produced, i e , subspecies *mutomoensis* produced the most seed (284 7 kg/ha), followed by *ethiopica* (244 0 kg/ha), *gibbosa* (213 3 kg/ha) and *nairobensis* (193 4 kg/ha)

Table 13 Days to flowering, harvesting period, and seed and oil yields of *Vernonia galamensis* growing at Kabete

Subspecies/ varieties	Days to 50% flowering	Harvesting period (days)	Yield (kg/ha)	Average oil yields (kg/ha)
Mutomoensis	103	118	475	147 1
Gibbosa	116	118	378	127 4
Ethiopica	129	104	351	108 6

Table 14 Average seed and oil yields of *Vernonia galamensis* grown at Kibwezi

Subspecies/ variety	Yield (kg/ha)	Mean oil yields (kg/ha)
Ethiopica	244 0	75 4
Nairobensis	193 4	62 3
Gibbosa	213 3	71 9
Mutomoensis	284 7	88 2

Results on the amounts of oil in both lipase-deactivated and lipase-nondeactivated vernonia seeds are presented in the Table 15 Generally, subspecies *gibbosa* had the highest amounts of oil while subspecies *mutomoensis* had the least

Table 15 Oil yields of deactivated and non-deactivated seeds of *Vernonia galamensis*

Subspecies/ variety	Deactivated seeds (g)	Percentage oil yield	Non-deactivated seeds (g)	Percentage oil yields
Nairobensis	56 5	14 9	20 0	12 1
Gibbosa	110 0	17 3	30 0	17 3
Mutomoensis	90 0	11 1	33 0	11 2
Ethiopica	60 0	11 6	45 2	11 8

The yield data obtained in Kenya this year and last year (see, Mills et al 1997) are relatively low This emphasizes the necessity for further genetic improvement and use of advanced agrotechnologies, such as sprinkle or drip irrigation

CHEMICAL STUDIES

INTRODUCTION

The constant increase in global demand for renewable resources for the chemical industry has spurred the search for new raw materials for the production for a variety of end uses New seed oil crops, such as Vernonia galamensis, can serve as one such renewable resource of chemical starting products for the production of a range of products, particularly surfactants The seeds of this species contain more than 40% oil, of which the vernolic (cis-12,13-epoxy-cis-9-octadecanoic) acid content is 72-81% (Carlson et al , Ayorinde et al 1988, 1990, Grinberg et al 1994) The unique structure of trivernolin and of its methyl ester enables us to perform a wide variety of reactions characteristic to the ester group, to the double bond, and to the epoxy group

In the past few years, our group has dealt with the preparation of new derivatives of vernonia oil as intermediates for the chemical industry by exploiting the unique structure of vernonia molecule

1 Bromo derivatives were prepared by addition and allylic bromination of the methyl ester of vernonia oil and of the oil itself

2 Vernonia oil was reacted with aliphatic diamines The study included the isolation and characterization of the intermediate—the monoaminoamide of vernolic acid—and the final product—N,N'-alkylbis (vernolamide) Recently, we developed improved procedures to obtain high yields of both the mono- and the corresponding divernolamides Fatty bisamides, which have a higher lubricating efficiency than primary amides, are used as slip agents, they also find application as corrosion inhibitors Diepoxy diamides contain two epoxy groups in the molecule, they thus can be used as a new class of reactive difunctional monomers with readily available amide blocks

3 The interaction of aromatic diamines with vernonia oil resulted in the production of a series of new rubber-like polymers

4 Acrylation and methacrylation of vernonia oil gave reactive α-olefinic monomers We are currently characterizing the monomers chemically and studying their thermal and photochemical polymerization

Our most up-to-date results on all these topics will be presented in our final report

As a part of our ongoing study on the applications of products based on vernonia oil, we directed the research described in this report to the production of vernolic salts and diethanolamides under conditions that facilitated the integrity of the epoxy ring The aim of the study was to produce novel surfactants from vernonia oil, since it is known that fatty acid salts (Sonntag 1979, Pryde 1979) and alkanolamides (Schich 1967) constitute a substantial portion of commercial anionic and nonionic surfactants

RESULTS AND DISCUSSION

1 VERNOLIC ACIDS AND THEIR SALTS

The conventional synthesis of fatty acids derived from natural oils is performed via the hydrolysis of glycerides (Sonntag 1979, Pryde 1979) We obtained a mixture of fatty acids of vernonia oil by the method described by Smith et al (1950), as follows Vernonia oil was treated with an aqueous ethanolic solution of potassium hydroxide at the reflux temperature for about 30 min After removal of the unsaponified matter and acidification, the solution was extracted with ether to give a mixture of fatty acids with the distribution

Vernolic acid	72-78%
Linoleic acid	9%
Oleic acid	3%
Stearic acid	2%
Palmitic acid	3%

Figure 1 shows the IR spectrum of the mixed fatty acids obtained from vernonia oil The following main absorption bands are found C-O-C stretching of the epoxy group at 824 and 845 cm ^{1}, carbonyl stretching at

1709 cm ^{1}, and a broad OH band stretching in the range 3100-3600 cm ^{1} The data were identical with those reported previously (Krewson et al 1962) The broad band in the range 3100-3600 cm^{-1} presumably incorporated both the acidic OH stretching and the diolic OH stretching formed to some extent by cleavage of epoxy group in the process of acid production

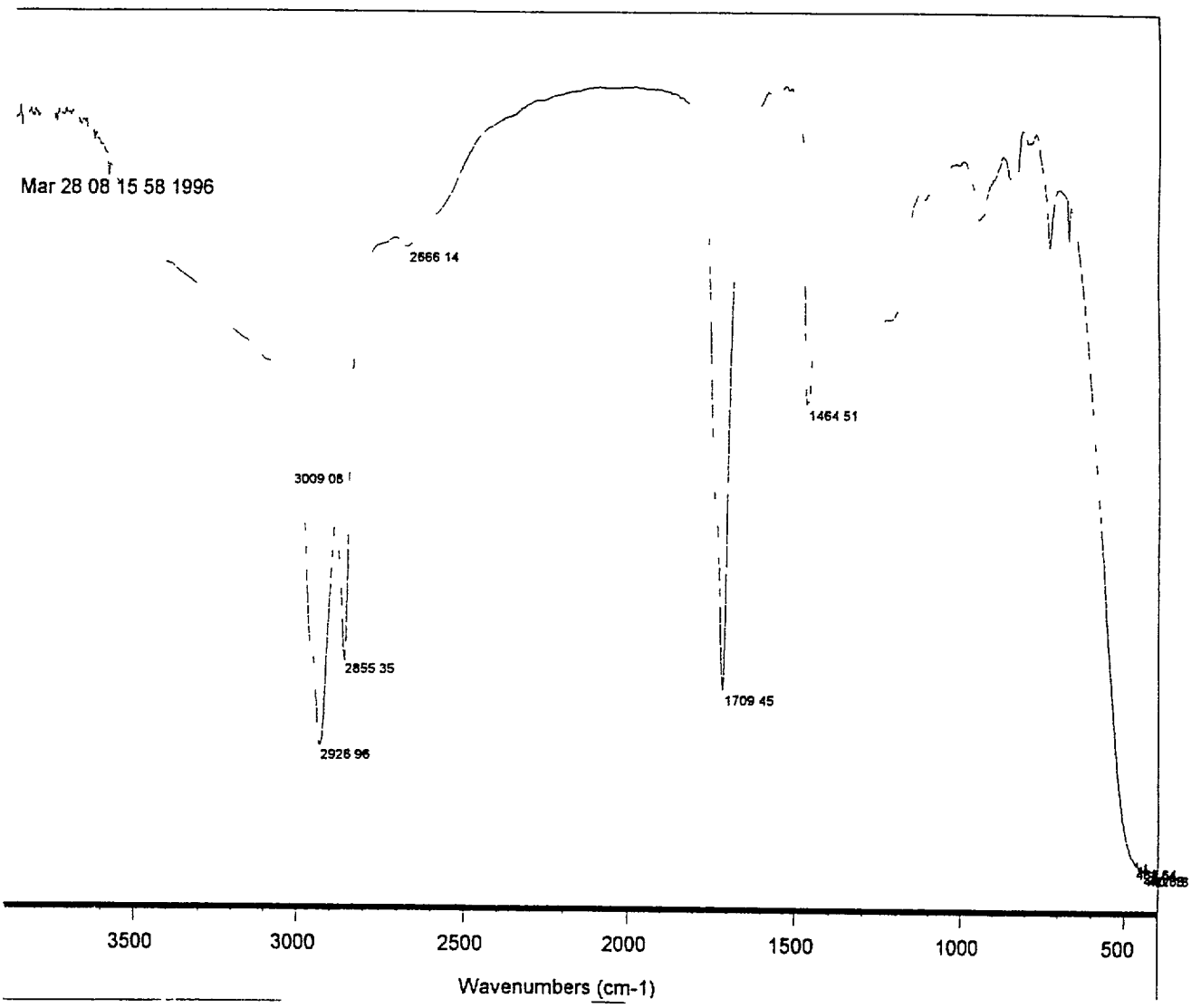
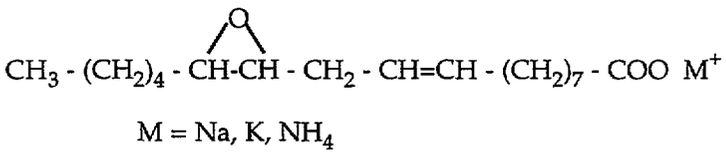


Figure 1 IR spectrum of the mixed fatty acids obtained from vernonia oil

2 SALTS OF VERNOLIC ACIDS

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)

Salts of vernolic acid and "dihydroxy vernolic" acid have not been reported in the literature The salts of vernolic acid were prepared by reacting the corresponding methyl esters with sodium or potassium hydroxide in absolute methanol or a methanol/hexane mixture as well as from the corresponding acids and potassium hydroxide in absolute methanol or sodium carbonate in a hexane/water mixture On the assumption that salts would be obtained in high yields by reacting equimolar quantities of the reagents to form water-soluble products, the salts were produced in this study by using a 5% deficit of alkali or sodium carbonate to assure complete conversion of these latter compounds and to facilitate further cleaning from unreacted starting material Sodium 12,13-dihydroxy-cis-9-octadecanoate was prepared by the reaction of the acid with sodium ethoxide in absolute ethanol



Vernolic acid salts

2 1 Analysis of the salts of vernolic acids

The analysis of epoxy fatty salts by the method of Jay (1964) showed that the titrating solution, a solution of hydrobromic acid in acetic acid, reacted not only with the oxirane function, but also with the carboxylic acid salt Titration data are given in Table 1 Purity of the salt relative to the metal (determined by AAS- atomic absorbtion spertometry) which differs from the purity of the salts relative to vernolic acid (oxirane value), varies dramatically (Table 1, Run 5, 10 and 12) The greater the purity of the salt relative to the metal, the lower the purity of the salt relative to the oxirane oxygen The salts obtained were white to yellow-brown, did not melt on heating to 200°C, and produced a brown color when heated to 100-120°C It

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has long been known (Pryde 1979) that high-purity fatty salts can be obtained by extraction or recrystallization We used IR data for examination of the changes taking place in the salts during the various purification procedures

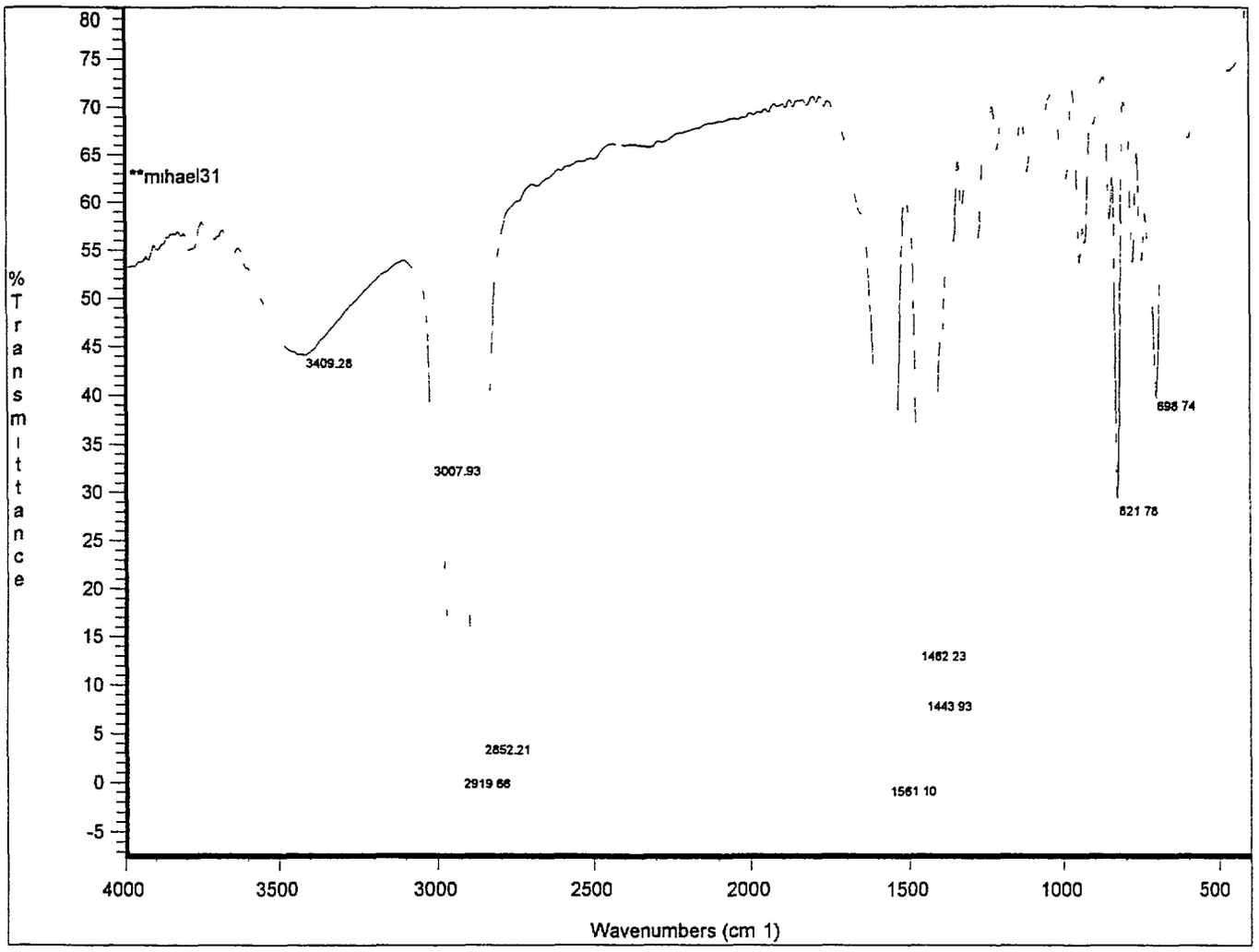


Figure 3 IR spectrum of sodium vernolic salts

Table 1 Properties of mixed vernolic salts and of sodium 12,13-dihydroxy-cis-9-octadecenoate

Run	Starting material	Salts: Metal, % Na[b]	Salts: Metal, % K[c]	Salts: Oxirane oxygen, %	Salts: Purity (%) relative to metal	Salts: Purity (%) relative to Vernolic
5	Vernolic esters	**6 4**	0 05	3 1	88 64	61 5
8	Vernolic acids	**7 3**	0 1	1 9	100 0	38 8
9	12,13-dihydroxy	**6 7**	0 02	-	98 1	-
10	Vernolic esters	0 09	**10 4**	2 9	88 9	60 7
		0 15	**10 4**	3 4	88 9	72 0
12	Methyl vernolate	0 09	**10 3**	3 7	88 1	78 8
13	Vernolic acids	0 07	**11 2**	2 6	95 8	54 4

Theoretical content of oxirane oxygen in sodium vernolate 5 02%
Theoretical content of oxirane oxygen in potassium vernolate 4 8%
Theoretical content of Na in vernolic salt is 7 22% and in 12,13-dihydroxy-cis-9-octadecenoate is 6 83%
Theoretical content of K in vernolic salt is 11 69%

The following absorption bands were evident in the IR spectra of all the salts examined (Figures 3-5) the band at 1560-1570 cm ^{1} (asymmetric COO^{-} vibrations), the broad band in the range 3100-3600 cm^{-1} with a maximum at 3430 cm ^{1} for sodium or potassium vernolic salts or at 3395 cm ^{1} for the non-epoxy salt XXII (the OH stretching), and the bands at 824 and 845 cm ^{1} (the C-O-C stretching of epoxy group) Extraction at room temperature or Soxhlet extraction of the raw salt with ether, petroleum ether, hexane or mixtures thereof removed unreacted starting methyl esters or acids However, the salts still contained impurities, as can be seen by the absorption in the 1610-1760 cm^{-1} region (Figures 3 and 4) The recrystallization of salts from water, methanol, or ethanol was not successful

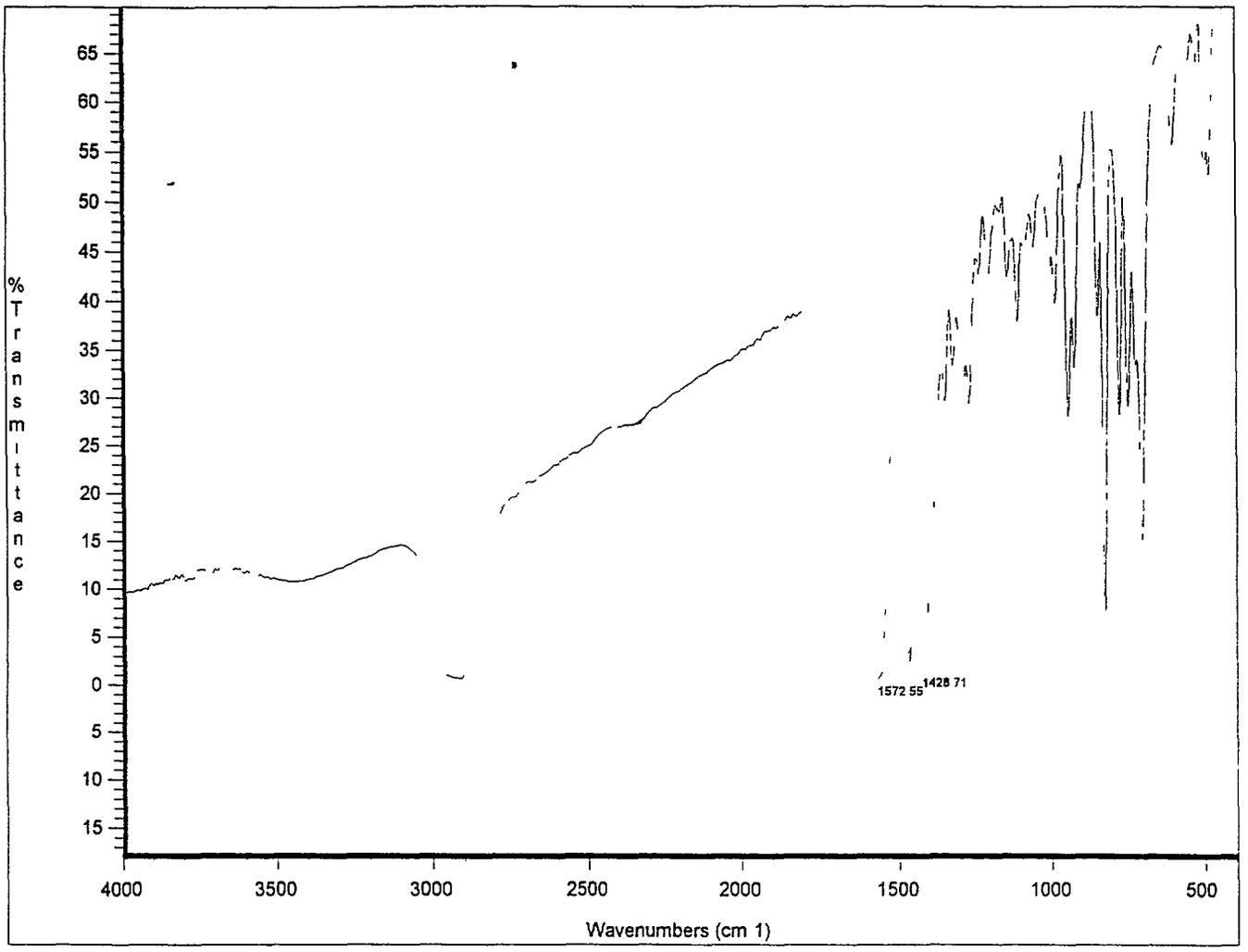


Figure 4 IR spectrum of potassium vernolic salts

2 2 Salt purification

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 (Figure 6)

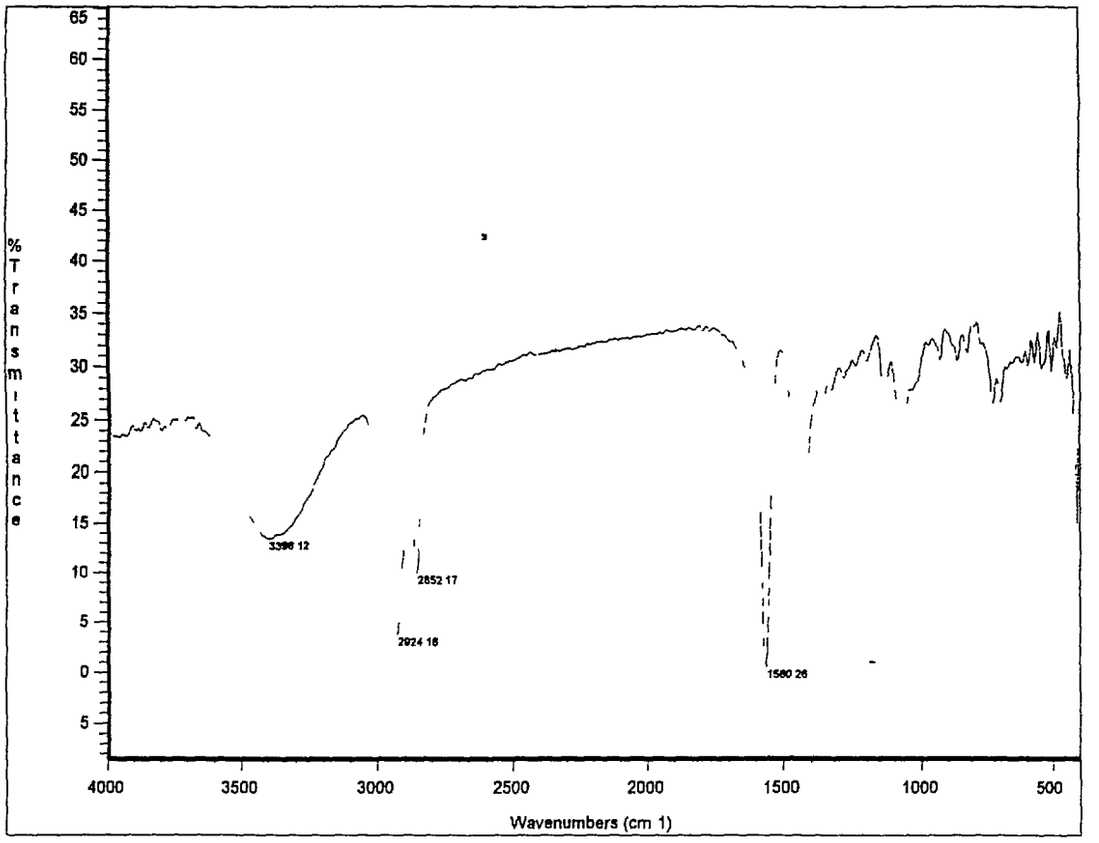


Figure 5 IR spectrum of sodium 12,13-dihydroxy-cis-9-octadecenoate

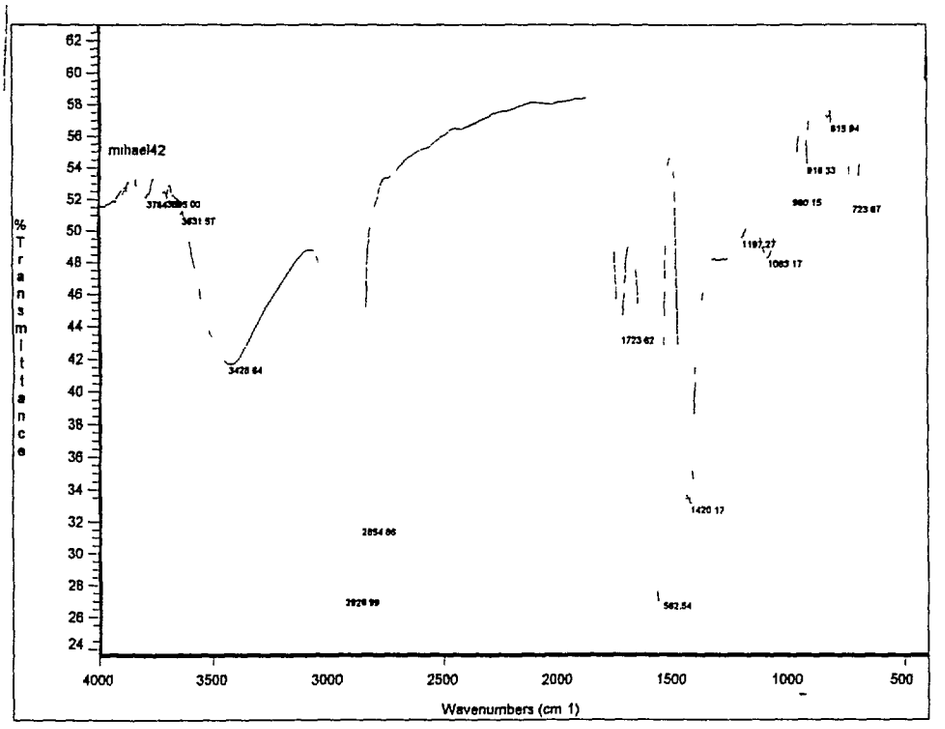


Figure 6 IR spectrum of sodium vernolic salts

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 (Figure 7) 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

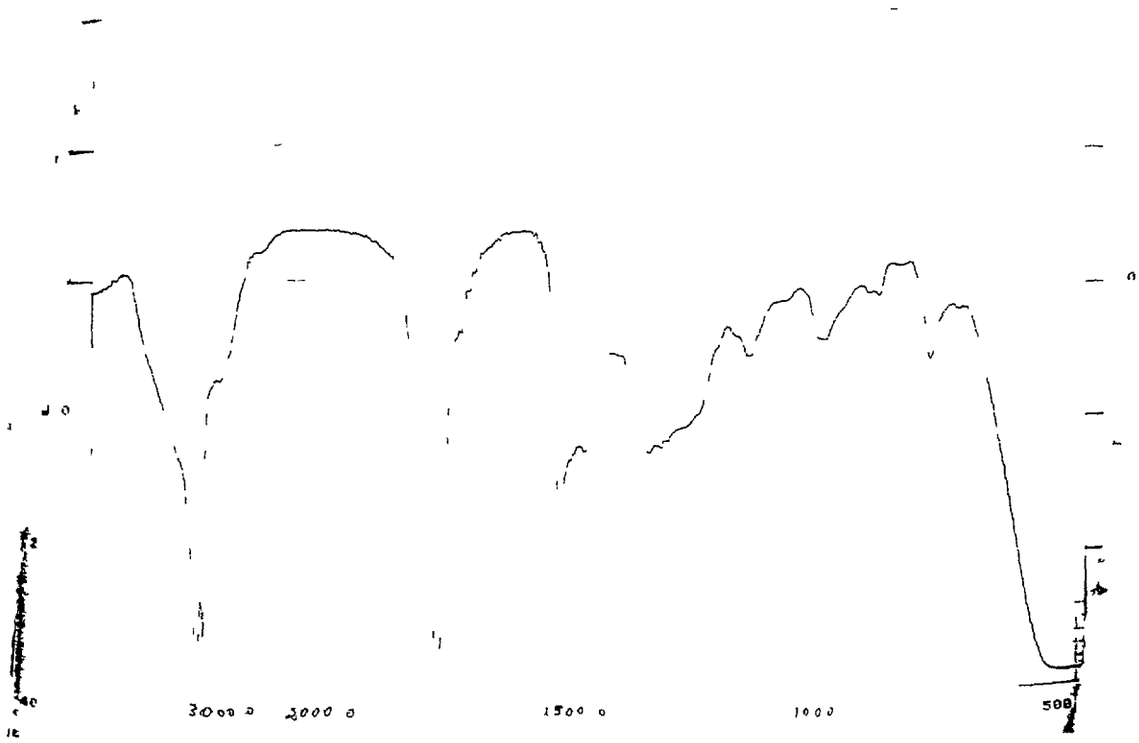


Figure 7 IR spectrum of mixed vernolic acids

3 Fatty alkanolamides

Secondary and tertiary alkanolamides are more commonly known as monoalkanolamides $RCONH(CH_2)_y(CHCH_3)_2OH$

or

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

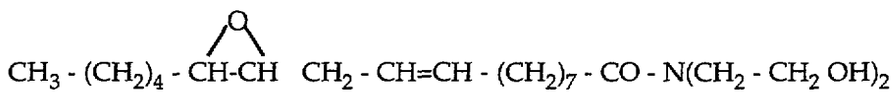
Monoalkanolamides are obtainable at 90% purity There are two classes of dialkanolamides the water soluble Kritcevsky-type amides (mixtures of 40-60% diethanolamide with diesters and unreacted diethanolamine) and the water-insoluble superamides (90-100% pure diethanolamide) The superamides comprise over 90% of the market for fatty alkanolamides, which are widely used versatile surfactants Foam stabilization and detergency are their most important functional properties In addition they are very effective in increasing the viscosity of detergent solutions and they provide emolliency, corrosion inhibition, emulsification wetting and lime-soap dispersion Diethanolamides find application in liquid dishwashing detergents, shampoos, lubricating oils and dry cleaning detergents as well as in textile processing

4 Vernolic diethanolamides

The synthesis of N,N-bis(2-hydroxyethyl)vernolamide has not been described in the literature Bryant et al (1993) reported the synthesis and isolation of epoxy secondary amides The reaction of vernonia oil with n-butyl-, n-pentyl- and n-hexylamine as the reagent and reflux solvent (i e , at 70-133°C) was completed in 1-5 h Yields of epoxy-containing amides were about 80%, with purity exceeding 96% in all cases However, N,N-bis-(2-hydroxyethyl) tallowamide was not formed from tallow and diethanolamide on heating at 50-60°C for 3-12 h (Feairheller et al 1994) It is also known (Feairheller et al 1994, Gast et al 1966, Jordan and Port 1961, Monick 1962) that the alkoxide-catalyzed aminolysis of normal vegetable oils or their methyl esters produces the corresponding amides in high yield

We previously reported the preparation of fatty acid bisamides containing the epoxy group in the molecule and of the corresponding monoamido amine by reacting vernonia oil with the relevant aliphatic diamines (Grinberg et al 1994) As is evident from the foregoing discussion, vernolic derivatives are most suitable for reactions under basic conditions, i e , with

retention of the epoxy ring We thus prepared vernolic diethanolamides from vernonia oil or the corresponding methyl esters and diethanolamine in the presence of sodium methylate as a catalyst The reaction was conducted at 110°C with molar proportions of oil/amine/catalyst 1 6 0 07 (35 min heating) or ester/amine/catalyst 1 2 0 024 (1 h heating) The reaction proceded with retention of the epoxy ring (oxirane oxygen titration, IR and NMR spectra), the yield usually being over 90% The high-purity N,N-bis(2-hydroxyethyl)vernolamide was isolated by column chromatography with a yield 75 8%



N,N-bis(2-hydroxyethyl) vernolamide

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), 1620 cm^{1} (the amide carbonyl stretching) and a broad band with the maximum at 3388 cm^{1} (OH stretching) The spectra of mixed vernolic diethanolamides obtained from oil or esters were identical These spectra differ from those 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 (Figure 8)

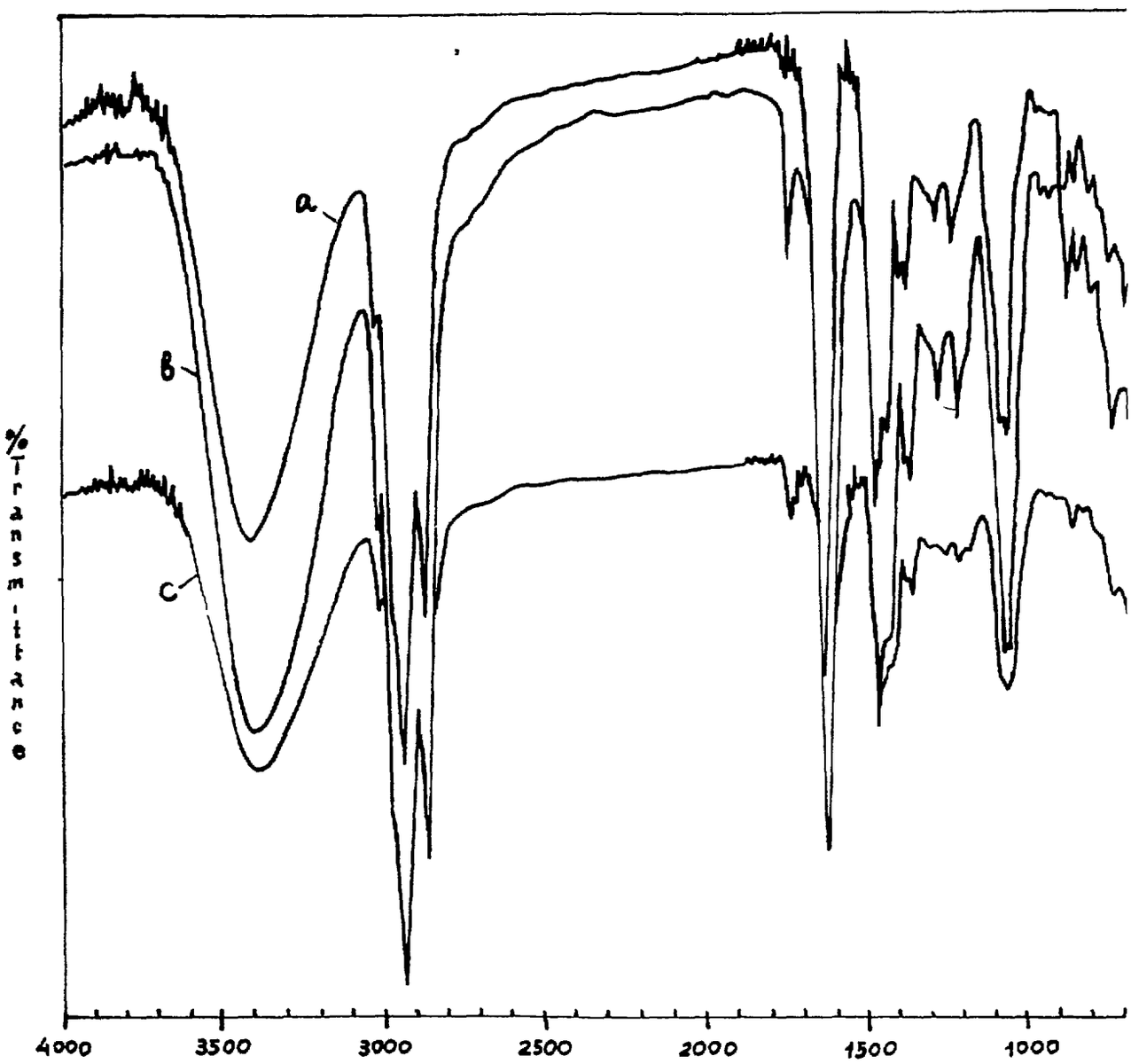


Figure 8 IR spectrum of N,N-bis(2-hydroxyethyl) vernolamide

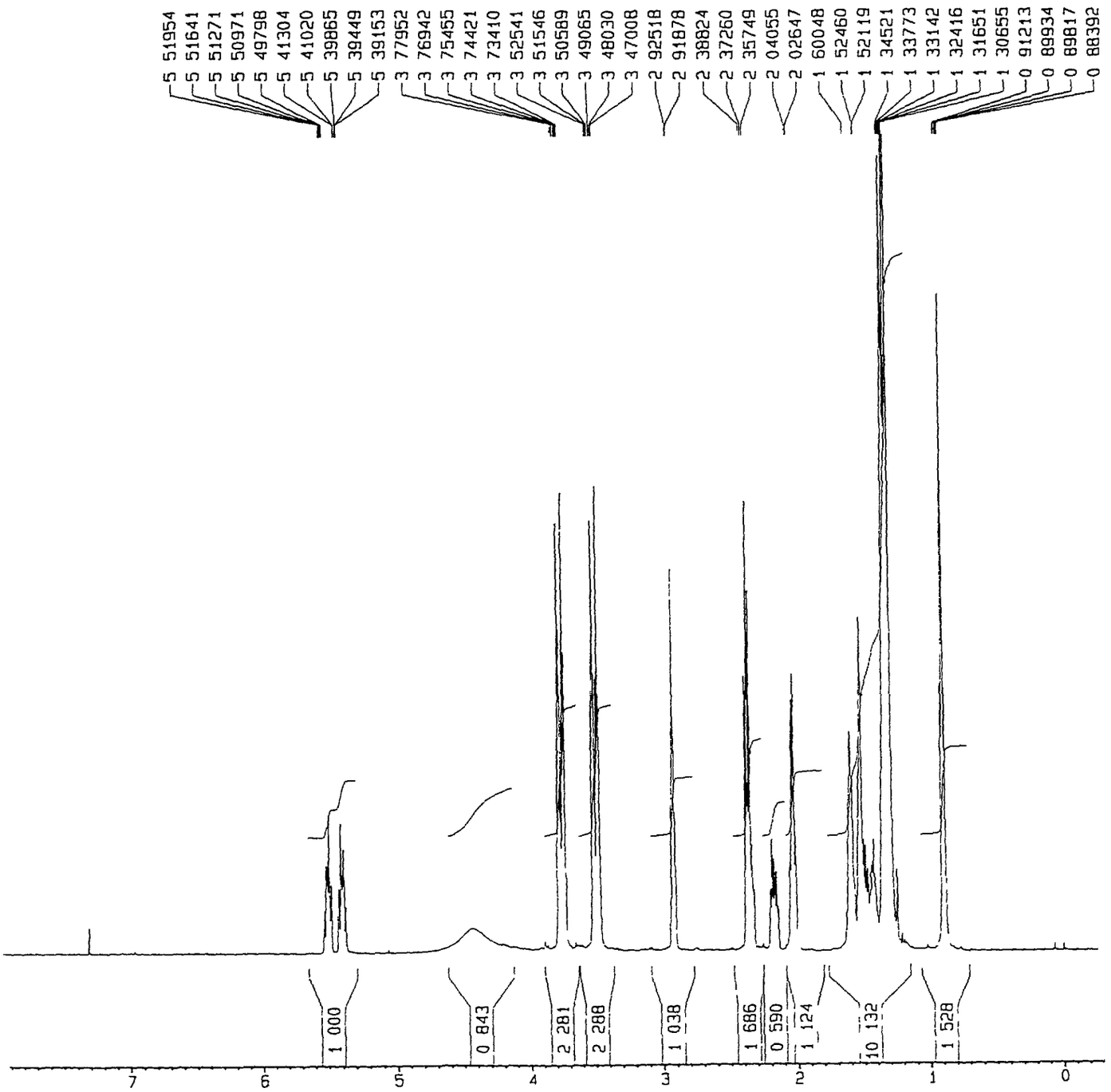


Figure 9 ^{1}H-NMR spectrum of vernolic diethanolamide

The ^{1}H-NMR spectrum of vernolic diethanolamide gave signals at δ (ppm) 0 9 (t, 3H, CH_3-18), 1 0-2 5 (complex signals, 24H, 12CH_2), 2 92 (m, –HC–CH–), 3 4-3 6 (m, 4H, 2CH_2OH), 4 0-4 8 (s, broad, 2H, 2OH) and 5 3-5 6 (complex signal, 2H, –HC=CH–) (Figure 9) ^{13}C-NMR peaks confirmed the presence of epoxy (56 48 and 57 15 ppm), olefinic (123, 65 and 132 43 ppm) and amide carbonyl (175 29 ppm) carbons (Figure 10) The vernolic amide also contained peaks at 13 81 ppm (CH_3), a series of peaks in the range from 22 38 to 31 52 ppm (CH_2 vernolic backbone), as well as peaks at 50 29 and 51 99 ppm (N-CH_2) and at 60 42 and 60 93 ppm (CH_2OH)

5 Solubility in water

The solubility data obtained in this study are given in Table 2 In solution, vernolic amides form milk-like liquids (suspension) that are difficult to separate This contributes to measurement errors The acid A_1 in Table 2 was obtained from vernonia oil by saponification with aqueous ethanolic sodium hydroxide (90°C, 1 h) and acidification to pH 4-5 The IR spectrum of this acid is given in Figure 1

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), e g , 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

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Table 2 Solubility data (g solute per 100 mL aqueous solution at room temperature)

	Acids	Esters	Salts			Amides	
Sample	Ac_1	E_1	S_1	S_2	S_3	Am_1	Am_2
Solubility	0 08	0	9 05	9 98	0 55	0 20	1 73

6 Critical micelle concentration

In surfactant solutions, aggregations of molecules (micelles) form in a rather narrow concentration range known as the critical micelle concentration (CMC) The CMC of surfactant indicates that monolayer absorption is complete and the surface active properties are optimal (Porter 1964) The CMC of diethanolamides Am_1 and Am_2 were calculated by the iodine solubilization method of Ross and Olivier (1959) The correlation of the CMC of our amides with the data from the literature is presented in Table 3 We should point out that diethanolamides Am_1 and those described in the literature (Malik and Chand 1966) were obtained by using similar reaction conditions

Table 3 Critical micelle concentrations (CMC) of fatty diethanolamides

Surfactant	Mol wt	t, °C	CMC			
			Procedure	wt %	mol/L	Reference
Am_1	383 58	22	Spectrophotometry	0 0016	4 05 x 10^{5}	Our work
Am_2		28	Spectrophotometry	0 0023		Our work
Lauroyl diethanolamide	287 45	25	Polarimetry, spectrophotometry	0 0011[a]	4 0 x 10^{5}	Malik and Chand 1966

7 Foaming

The foaming properties of two vernolic derivatives (anionic and nonionic), determined by the standard method (Ross and Miles 1941) in both pure (deionized) water and hard water (total hardness 1,000 ppm as $CaCO_3$), are given in Table 4 The results correlate with those described in the literature In pure water, anionic vernolic salts S_3 produced more foam and the foam was more stable than nonionic vernolic diethanolamides Am_2 The lower foaming of S_3 in comparison with typical high-foaming anionic surfactants

SDBS and SLS (Table 4) is related to their larger alkyl chain length, since maximum foam production occurs with soaps made from C-12 to C-14 fatty acids (Porter 1964) Vernolic diethanol amides Am_2 have foaming ability in hard water, and the same is true for other fatty diethanolamides (Schick 1967, Porter 1964)

Table 4 Foaming properties of vernolic surfactants

Compound	Property	Foam height, mm				Reference
		Initial		After 5 min		
		Pure water	Hard water	Pure water	Hard water	
S_3	Anionic	90	a	15	a	Our work
SDBS[b]	Anionic	160		140		Schick M J (ed) 1967
SLS[c]	Anionic	160		140		Schick M J, (ed) 1967
Am_2	Nonionic	13	8	9	2	Our work
POENP[d]	Nonionic	15-130		10-110		Schick M J (ed) 1967

[a]A precipitate is obtained solution in hard water, [b]SDBS sodium dodecylbenzene sulfonate, [c]SLS - Sodium lauryl sulfate, [d]POENP Polyoxyethylene nonylphenols

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