The Study of Natural Epoxy Oils and Epoxidized Vegetable Oils by ^{13}C Nuclear Magnetic Resonance Spectroscopy

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The ^{13}C nuclear magnetic resonance spectra of *Vernonia galamensis* seed oil and of epoxidized palm super olein, soybean oil and linseed oil have been recorded and interpreted. The chemical shifts of the major signals are assigned and semi-quantitative results are derived. The spectroscopic procedure provides a useful method of analyzing oils that contain epoxy acids. The epoxide function differs from a double bond in its influence on the chemical shifts of nearby carbon atoms.

KEY WORDS: ^{13}C nuclear magnetic resonance spectra, epoxidized linseed oil, epoxidized oils, epoxidized palm super olein, epoxidized soybean oil, *Vernonia galamensis* seed oil.

There are two areas of commercial interest in epoxy oils—oils that contain natural epoxy acids and epoxidized oils produced and used on an industrial scale. Following the discovery of vernolic acid (*cis*-12,13-epoxyoleic) as the first natural epoxy acid (1), other acids of this class were recognized (2), but vernolic acid is still the only natural epoxy acid of potential value. It occurs at high levels in sources, such as the seed oils of *Vernonia anthelmintica* (72%), *V. galamensis* (73–78%), *Stokesia laevis* (65–79%), *Cephalocroton cordofanus* (62%), *Euphorbia lagascae* (57–62%), *Erlangea tomentosa* (52%), *Crepis aurea* (52–54%) and *C. biennis* (68%), and there is a steady flow of papers on the potential value of oils containing this acid (3–12).

Epoxidized oils—especially soybean and linseed, but also palm oil—are produced on an industrial scale ($\approx 10^8$ lb/yr) for use as stabilizers and plasticizers in polyvinyl chloride. The composition of these products follows from that of the starting material, assuming that it is completely epoxidized and there is no residual unsaturation. Oleic, linoleic and linolenic esters are thus converted to mono-, di- and triepoxides. Total oxirane (epoxide) content can be measured, but there has been little or no attempt to analyze the products in terms of their various epoxy acids.

Epoxidation is a stereospecific (*cis*) addition process, and because the starting olefin (such as the unsaturated vegetable oils) has *cis* configuration, the epoxide will be *cis* as well. Nevertheless, questions of stereochemistry have to be considered. Oleic esters give two enantiomeric epoxystearates, which appear as a single racemic (*cis*) form (elaidic ester would furnish the *trans* racemate). Linoleate gives two diastereoisomeric racemates (all *cis*) from the four possible enantiomers, and linolenate gives four diastereoisomeric racemates (all *cis*) from eight possible enantiomers. The diastereoisomeric racemates are not necessarily formed in equal amounts.

Some information on the ^{13}C nuclear magnetic resonance spectra of epoxy esters is given in a paper by Bascetta and Gunstone (13), who studied several *cis* and *trans*-epoxyoctadecanoates and some *cis*-epoxyoctadecenoates (*cis* isomers), including methyl vernolate. They concluded that in the saturated esters epoxy carbon atoms gave a signal around 58.5 ppm and that the *cis* epoxide group had an influence on the chemical shifts of the α (−1.7), β (−2.9) and γ (−0.4) carbon atoms. It seems likely that a CH_2 group α to two epoxide groups will have its chemical shift changed by around 2×-1.7 and, therefore, will not be very different from CH_2 groups β to one epoxide group. For a carbon atom between one epoxide function (−1.7) and one double bond (−2.5), the change is only −3.2, and between two double bonds only −4.2, so these influences are not fully additive.

EXPERIMENTAL PROCEDURES

The sample of *V. galamensis* seed oil was supplied some years ago by Dr. S. Crook of Castrol International. Dr. P. W. R. Smith (also of Castrol International, Pangbourne, England) provided the epoxidized palm super olein. The epoxidized soybean oil (Lankroflex GE) and epoxidized linseed oil (Lankroflex 1) were provided by J.T. Connal of Harcros Chemical Group (Manchester, England). Spectra were obtained with a Bruker AM 300 spectrometer (pulse angle 45°, pulse repetition line 1.82 s, resolution 1.22 Hz per data point, ≈1000 scans), (Bruker Spectrospin, Burlington, Ontario, Canada) with solutions in $CDCl_3$.

RESULTS AND DISCUSSION

Vernonia oil. Data for the spectrum of *V. galamensis* seed oil is presented in Table 1. The spectrum has 63 signals, but it is not too difficult to interpret in terms of an oil rich in vernolic acid (<70%) and also containing linoleic, oleic and saturated acids.

The spectrum shows expected signals for C1–3 and for glycerol. Some minor signals for C2, C3 and glycerol carbon atoms are not assigned. The olefinic section of the spectrum shows large signals associated with vernolate at 132.40 (V10) and 124.05 ppm (V9), smaller signals for oleic and linoleic esters, and three still smaller signals, which are not assigned. There are five epoxide signals: three small ones, which are not identified, and the two major signals at 57.05 (V12) and 56.40 (V13), which are associated with vernolate. The minor signals suggest the presence of other epoxy esters at low levels. Although other natural epoxy acids are known, none has been reported in vernonia oil.

The ω1–3 signals provide useful information. In addition to major signals for V18 (14.01), V17 (22.61) and V16 (31.77), there are signals expected from saturated and from n-9 and n-6 unsaturated esters, along with some additional small and unassigned signals.

The 13 signals between 28.1 and 25.3 ppm are the most difficult to interpret. They are from carbon atoms that are α to the double bond (allylic) or α or β to the epoxide function. The four intense signals must be associated with vernolate: 27.79 (V14), 27.40 (V8), 26.33 (V11) and 26.28 (V15), but the V14 and V8 signals may be interchanged, as may those assigned to V11 and V15. The next two most intense signals are associated with oleate and linoleate (Table 1), leaving seven smaller signals not assigned.

Because the spectrum was not collected under conditions to give quantitative results, the intensities can only be used for this purpose in an approximate manner. With this safeguard in mind, the ω1–3 and the olefinic

TABLE 1

^{13}C Nuclear Magnetic Resonance Spectrum of Vernonia Oil

Assignment[a,b]		δ (ppm)	Intensity
C1	α	173.01	6.55
	β	172.62	3.86
C2		34.68	0.74
		34.53	0.30
		34.32	0.21
	β	34.13	6.53
	α	33.97	11.88
		33.74	0.20
C3		24.83	15.28
		24.67	0.20
ω3		32.25	0.23
	n-9, sat	31.94	2.40
	V16	31.77	20.95
		31.61	1.08
	n-6	31.54	3.22
ω2		22.81	0.39
	n-9, sat	22.71	3.18
	V17, n-6	22.61	21.40
		22.38	0.20
		20.70	0.28
ω1	n-9, sat	14.12	4.03
	n-6	14.08	3.56
	V18	14.01	18.03
Olefinic	V10	132.40	14.12
		132.11	0.25
	L13	130.09	2.31
	L9, O10	129.90	2.20
		129.77	0.20
	O9	129.66	0.65
		128.43	0.69
	L10	128.06	2.59
	L12	127.91	2.88
	V9	124.05	15.67
		115.86	0.35
Glycerol	β	68.95	5.05
		65.25	0.26
		63.01	0.18
	α	62.07	9.90
Epoxidic carbon	V12	57.05	17.66
		56.80	0.24
		56.70	0.21
	V13	56.40	17.86
		56.10	0.20
Carbons α and β to epoxide and allylic		28.07	0.25
	V14[c]	27.79	21.08
		27.60	0.64
	V8[c]	27.40	17.57
	O8, 11; L8, 14	27.20	5.89
		26.93	1.08
		26.54	0.49
	V11[d]	26.33	22.69
	V15[d]	26.28	20.20
		26.09	0.42
		25.99	0.24
	L11	25.64	2.59
		25.29	0.59

[a] Assignments; C1–3 and ω1–3 refer to carbon atoms at the acyl and methyl ends of the chain, respectively. O, oleic; L, linoleic, V, vernolic, sat, saturated.

[b] Also methylene envelope 29.72–29.10 (7 signals).

[c,d] These signals could be interchanged.

TABLE 2

Composition of Vernonia Oil from Its ^{13}C Nuclear Magnetic Resonance Spectrum (mol % based on intensities in Table 1)

Acyl chain	ω3	ω2	ω1	Olefinic[a]
Saturated	8.6	12.5	15.7	—
n-9				3.6
Other	4.7	3.4		4.0
n-6	11.5	84.1	13.9	12.6
Vernolate	75.2		70.4	79.8

[a] Olefinic signals give information about unsaturated acyl chains only.

signals give the results summarized in Table 2 and indicate the presence of vernolate (70–75%), linoleate (≈13%) and oleate (≈4%).

Epoxidized seed oils. Three epoxidized oils were examined spectroscopically—epoxidized palm super olein (Ep PSO), epoxidized soybean oil (Ep SB) and epoxidized linseed oil (Ep Lin). These were expected to be rich in mono-, di- and triepoxides, respectively, and this fact has been used in interpreting the spectra. The three spectra contain 45, 68 and 59 signals, respectively. The results, apart from signals for the glycerol carbon atoms and those in the methylene envelope (29.1–29.7 ppm), are given in Tables 3–8. It has already been indicated that the mono-, di- and triepoxystearates exist in 1, 2 and 4 diastereoisomeric pairs and that, where more than one diastereoisomer exists, these may not be formed in equal amounts. Also, because the number of signals is less than expected, some of them must overlap. Despite the complexity of the spectra, valuable information can be obtained.

A total of 21 epoxide signals with chemical shifts between 58.3 and 53.2 ppm are apparent between the three spectra (Table 3). The Ep PSO shows two large signals at 57.18 and 57.13 ppm, probably linked to C10 and C9 of the 9,10-epoxystearates, and six smaller signals. On the basis of unpublished data on epoxidized methyl linoleate (kindly supplied by Smith of Castrol International), the diepoxystearates have eight signals, including two that overlap with the signals from the 9,10-epoxystearates. From the intensities of these signals and of the major signals observed with Ep SB, it follows that the two stereoisomeric pairs (designated A and B) have the shifts shown in Table 4 and that the stereoisomeric pair B is produced in larger amounts than stereoisomeric pair A. Ep Lin has 18 signals—13 large, 2 medium and 3 small. The larger signals must be associated with triepoxystearates (24 possible signals). These must overlap with one another and with signals from the mono- and diepoxystearates, and it is not possible to assign them further. None of the spectra show any olefinic signals, indicating that epoxidation is virtually complete.

The three spectra show 14 signals in the region 26.1–29.0 ppm, as well as some smaller signals in the region 25.6–28.8 (Table 5). These result from carbon atoms α, β or γ to one or more epoxide groups, and the actual carbon atoms involved are set out in Table 6. On the basis of the effect of an epoxide group on nearby carbon atoms (as previously discussed), it is likely that in Ep PSO the large peaks at 28.99, 27.85 and 26.63 are from carbon atoms γ(C6, C13), α (C8, C11) and β(C7, C12) to the epoxide function. Smaller signals at 27.25, 26.96, 26.49, 26.27 and 26.16, all of which are larger in the Ep SB spectrum, must be associated with diepoxystearates and especially with C11, C14 and C15. Again, the stereoisomers may give different chemical shifts. It is reasonable to conclude that signal 1 refers to carbon atoms γ to an epoxide function, signals 2–10 relate to carbon atoms α to an epoxide function and signals 11–14 relate to carbon atoms β to an

TABLE 3

Epoxide Carbon Atoms—Chemical Shifts and Intensity[a]

Signal number	Ep PSO δ (ppm)	Ep PSO Intensity	Ep MeLin[b] δ (ppm)	Ep SB δ (ppm)	Ep SB Intensity	Ep Lin δ (ppm)	Ep Lin Intensity
1	—	—	—	—	—	58.29	0.76
2	—	—	—	58.13	0.74	58.13	6.35
3	—	—	—	57.83	0.47	57.83	4.49
4	57.18	6.45	57.24	57.19	7.85	57.18	6.47
5	57.13	6.11	57.20	57.13	7.11	57.13	6.14
6	56.99	1.30	57.03	56.99	10.53	56.98	5.05
7	56.93	1.15	56.98	56.93	11.15	56.92	10.48
8	56.71	0.69	56.74	56.71	6.31	56.71	2.95
9	56.63	0.59	56.68	56.64	6.38	56.63	6.46
10	—	—	—	56.51	1.27	—	—
11	—	—	—	—	—	56.41	0.76
12	—	—	—	—	—	56.27	0.85
13	54.33	1.72	54.37	54.33	15.31	54.32	6.59
14	—	—	—	—	—	54.24	9.16
15	54.17	1.16	54.22	54.17	14.53	54.17	24.40
16	—	—	—	—	—	54.09	12.55
17	—	—	—	—	—	54.05	9.92
18	—	—	—	53.97	1.33	53.97	6.60
19	—	—	—	53.78	0.58	53.77	3.47
20	—	—	—	53.31	0.34	—	—
21	—	—	—	53.23	0.39	—	—

[a] EP PSO, epoxidized palm super olein; Ep MeLin, epoxidized methyl linoleate; Ep SB, epoxidized soybean oil; Ep Lin, epoxidized linseed oil.

[b] Chemical shift data supplied by Dr. P.W.R. Smith (Castrol, Pangbourne, England), also a signal at 56.55 ppm.

TABLE 4

Chemical Shifts (ppm) and Intensities of Epoxide Carbons in Diepoxystearate

	Diastereoisomer A		Diastereoisomer B	
Epoxidized oil	δ (ppm)	Intensity	δ (ppm)	Intensity
Palm super olein	57.18	—[a]	56.99	1.30
	57.13	—[a]	56.93	1.15
	56.71	0.69	54.33	1.72
	56.63	0.59	54.17	1.16
Soybean	57.19	—[a]	56.99	10.53
	57.13	—[a]	56.93	11.15
	56.71	6.31	54.33	15.31
	56.64	6.38	54.17	14.53

[a] Overlap with signal from 9,10-epoxystearate.

epoxide function or lying between two epoxide groups. It is not yet possible to take the assignments beyond this tentative stage.

Chemical shifts and intensities associated with C1-3 and ω1-3 are listed in Table 7. In the signals for C1 and C2, it is possible to distinguish α- from β-chains and epoxy from nonepoxy chains (14). All the epoxy esters have a 9,10-epoxide (at least) and are not distinguishable from each other. In all three of the epoxidized oils, there are three signals for carbon atoms 1 and 2: two for the α-chains (saturated and epoxy esters) and one for the β-chain (epoxy esters only). The difference in chemical shift between saturated and 9,10-epoxy esters is greater than between saturated and 9,10-olefinic esters. The distribution of saturated and epoxy chains is not surprising because it reflects the distribution of saturated and unsaturated chains in the original oil, and it is known that vegetable oils generally have little or no saturated ester in the β-position (15). The relative amounts of saturated and epoxide chain in the α-position indicate the higher level of saturated acids in the Ep PSO than in the epoxidized soybean and linseed oils.

The ω1-3 signals provide the best information about the properties of mono-, di- and triepoxides because it is possible to distinguish between 9,10-, 12,13 and 15,16-epoxides. It is assumed that these signals are indicative of mono-, di- and triepoxides, respectively. The ω1 signals for the three epoxide systems are at 14.12 (overlapping with nonepoxy esters), 14.00 and at 10.61 and 10.50 ppm, respectively. These conclusions are consistent with data for the 9,10- and 15,16-epoxystearates reported by Bascetta and Gunstone (13), with the results for vernonia oil reported in this paper and with the relative amounts of these epoxides expected in the three oils. The two signals for the 15,16-epoxides must be associated with the stereoisomeric forms of the triepoxide. The ω2 signals, identified in a similar way, are at 22.68 (saturated and 9,10-epoxides), 22.58 (12,13-epoxides) and 21.25 and 21.16 ppm (15,16-epoxides). The ω3 signals are identified for nonepoxy esters (31.93), 9,10-epoxides (31.86) and

TABLE 5

Carbon Atoms α, β or γ to an Epoxide System[a]

Signal number	Ep PSO		Ep SB		Ep Lin	
	δ (ppm)	Intensity	δ (ppm)	Intensity	δ (ppm)	Intensity
1	28.99[b]	5.91	28.99	16.94	28.97	18.70
2	—	—	27.90	19.27	27.90	15.14
3	27.85[c]	9.76	27.85	22.07	27.84	19.17
4	—	—	27.41	1.42	27.38	6.73
5	—	—	—	—	27.31	4.91
6	27.25[d]	1.17	27.24	12.03	27.25	8.26
7	—	—	—	—	27.18	5.03
8	—	—	—	—	27.05	4.52
9	26.96[d]	0.68	26.95	7.40	26.94	6.82
10	—	—	—	—	26.87	3.12
11	26.63[e]	11.34	26.62	15.46	26.60	14.03
12	26.49[d]	1.11	26.47	9.94	26.47	10.58
13	26.27[d]	0.72	26.26	7.39	26.25	3.29
14	26.16[d]	1.09	26.15	12.26	26.14	4.84
Other signals	28.79	0.30	28.68	0.62	28.76	0.93
	—	—	28.54	0.35	—	—
	—	—	28.11	0.59	—	—
	—	—	27.52	0.58	—	—
	—	—	27.06	0.86	—	—
	—	—	26.80	0.57	—	—
	—	—	26.01	0.68	—	—
	—	—	25.89	0.55	—	—
	—	—	25.67	0.43	—	—
	—	—	25.59	0.41	—	—

[a] Abbreviations as in Table 3.
[b] Carbon atoms γ (C_6 and C_{13}) to epoxide in monoepoxystearates.
[c] Carbon atoms α (C_8 and C_{11}) to epoxide in monoepoxystearates.
[d] Signals associated with C_{11}, C_{14} and C_{15} in diepoxystearates.
[e] Carbon atoms β (C_7 and C_{12}) to epoxide in monoepoxystearates.

TABLE 6

Carbon Atoms α, β and γ to Epoxide Groups in Mono-, di- and Triepoxystearate

	α	αα	β	γ
Mono-	8,11	—	7,12	6,13
Di-	8,14	11	7,15	6,(16)[a]
Tri-	8,(17)[a]	11,14	7(18)[a]	6

[a]These refer to ω1, ω2 and ω3 signals with different chemical shifts (see Table 7).

12,13-epoxides (31.68). The ω3 signal for 15,16-epoxides is also epoxidic and has already been considered (Table 3). The epoxidized soybean oil contains some additional minor signals, which have not been assigned.

Recognizing that the quantitative results will not be accurate because allowance has not been made for differences in relaxation times, which are known to increase at the ω end of the acyl chain, it is still interesting to use the ω1-3 signals to calculate the composition of these oils (Table 8). It must be remembered that the results are mol%, that saturated and 9,10-epoxides are not well separated, except for the ω3 signal, and that 15,16-triepoxides do not give useful ω3 signals. These last signals will, therefore, give high values for the remaining categories of acids. Taking all these facts into account, the approximate analysis (mol%) of the three epoxidized oils is: PSO, saturated 48, monoepoxide 41, di-epoxide 11, triepoxide nil; SB, saturated 16, monoepoxide 21, diepoxide 57, triepoxide 3; Lin, saturated 9, monoepoxide 18, diepoxide 24, triepoxide 49.

TABLE 7

Epoxidized Oils C1-3 and ω1-3 Signals—Chemical Shift (ppm) and Intensity[a]

	Ep PSO		Ep SB		Ep Lin	
Assignment	δ (ppm)	Intensity	δ (ppm)	Intensity	δ (ppm)	Intensity
C1						
α sat	173.23	3.11	173.24	2.70	173.24	1.83
α ep	173.16	1.72	173.16	6.94	173.15	7.79
β ep	172.75	1.89	172.75	4.82	172.75	4.87
C2						
β ep	34.16	3.92	34.14	8.80	34.13	8.55
α sat	34.05	6.34	34.05	6.97	34.03	4.96
α ep	34.00	3.63	33.98	13.27	33.97	14.77
C3						
—	25.03	0.33	—	—	—	—
—	24.88	7.92	—	—	—	—
—	24.84	6.18	24.80	17.31	24.78	18.07
—	—	—	24.55	0.48	—	—
ω1			14.24	0.50		
sat; 9,10 ep	14.12	9.64	14.12	9.00	14.11	7.51
12,13-ep	13.99	1.87	14.00	15.10	13.99	5.98
15,16-ep	—	—	10.61	0.63	10.61	5.79
15,16-ep	—	—	10.50	0.59	10.49	7.93
ω2						
sat	22.70	8.47	—	—	—	—
9,10-ep	22.68	8.76	22.68	8.51	22.67	7.53
12,13-ep	22.58	2.21	22.58	17.80	22.57	6.75
15,15-ep	—	—	21.25	0.62	21.24	7.13
15,16-ep	—	—	21.16	0.58	21.16	6.53
ω3						
sat	31.94	7.68	31.93	6.36	31.92	3.67
9,10-ep	31.88	6.65	31.86	8.07	31.85	6.87
—	—	—	31.78	1.36	—	—
12,13-ep	31.69	1.83	31.68	16.00	31.67	6.26
—	—	—	31.49	0.59	—	—
—	—	—	31.38	0.49	—	—

[a]Abbreviations as in Tables 1 and 3; ep, epoxy.

TABLE 8

The Composition (mol%) of Three Epoxidized Oils Based on Their ω1, ω2 and ω3 Signals[a]

	Ep PSO			Ep SB			Ep Lin		
	ω1	ω2	ω3	ω1	ω2	ω3	ω1	ω2	ω3
Sat	84	44	48	37	31	19	28	27	22
9,10-ep	—	45	41	—	—	25	—	—	41
12,13-ep	16	11	11	58	65	49	22	24	37
15,16-ep	—	—	—	5	4	—	50	49	—
Other	—	—	—	—	—	7	—	—	—

[a]Abbreviations as in Tables 1 and 3; ep, epoxy.

To obtain more accurate results, it would be necessary to measure T_1 and then to incorporate an appropriate relaxation time into the protocol for collecting the spectrum. This presents no insuperable problem, and a good spectroscopic method of analyzing epoxy oils could be derived.

ACKNOWLEDGMENTS

I thank M. Smith and H. Walshaw for technical assistance and acknowledge financial support provided by the Karlshamn Research Foundation. Dr. Crook, Dr. Smith and Mr. Connal supplied materials.

REFERENCES

1. Gunstone, F.D., *J. Chem. Soc.*:1611 (1954).
2. Gunstone, F.D., in *The Lipid Handbook*, edited by F.D. Gunstone, J.L. Harwood and F.B. Padley, Chapman and Hall, London, 1986, pp. 21, 52.
3. Carlson, K.D., W.J. Schneider, S.P. Chang and L.H. Princen, in *New Sources of Fats and Oils*, edited by E.H. Pryde, L.H. Princen and K.D. Mukherjee, American Oil Chemists' Society, Champaign, 1981, pp. 297–318.
4. Carlson, K.D., and S.P. Chang, *J. Am. Oil Chem. Soc. 62*:934 (1985).
5. Perdue, Jr., R. E., K.D. Carlson and M. Gilbert, *Econ. Bot. 40*:54 1986.
6. Watanabe, S., T. Fujita and M. Sakamoto, *J. Am. Oil Chem. Soc. 65*:1311 (1988).
7. Ayorinde, F.O., G. Osman, R.L. Shepard and F.T. Powers, *Ibid. 65*:1774 (1988).
8. Ayorinde, F.O., F.T. Powers, L.D. Streete, R.L. Shepard and D.N. Tabi, *Ibid. 66*:690 (1989).
9. Ofalabi, O.A., M.E. Aluko, G.C. Wang, W.A. Anderson and F.O. Ayorinde, *Ibid. 66*:983 (1989).
10. Ayorinde, F.O., B.D. Butler and M.T. Clayton, *Ibid. 67*:844 (1990).
11. Bryant, K.A.A., C.P. Nwaoicha, M.A. Anderson and F.O. Ayorinde, *Ibid. 69*:1023 (1992).
12. Yalley, E.S., F.O. Ayorinde and B.E. Eribo, *Ibid. 69*:1046 (1992).
13. Bascetta, E., and F.D. Gunstone, *Chem. Phys. Lipids 36*:253 (1985).
14. Lie Ken Jie, M.S.F., C.C. Lam and B.F.Y. Yan, *J. Chem. Res.* S12 and M250, (1992).
15. Gunstone, F.D., and F.A. Norris, *Lipids in Foods*, Pergamon, Oxford, 1983, p. 22.

[Received April 5, 1993; accepted August 11, 1993]