Bull. Chem. Soc. Ethiop. **2004**, 18(1), 59-65. ISSN 1011-3924
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HIGH FIELD ^{13}C NMR SPECTROSCOPIC ANALYSIS OF THE TRIACYLGLYCEROLS OF *JATROPHA CURCAS* OIL

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(Received April 28, 2003; revised September 17, 2003)

ABSTRACT. Gated decoupled ^{13}C NMR has been employed to determine the acyl composition and acyl positional distribution on the glycerol backbone in the triacylglycerols of *Jatropha curcas* oil. Results revealed the presence of saturated, oleic and linoleic acids in the oil. Integrals obtained from the allylic carbons were used for semiquantitative analysis of the oil and gave the percentage of total saturated, oleic and linoleic acids in the oil as 27, 41 and 32, respectively. The results obtained by ^{13}C NMR compared favourably with those obtained by gas chromatographic analysis. Analysis of the spectra further revealed that the saturated acyl esters are randomly distributed in the α and β glyceridic positions, with oleic being approximately 60% distrbuted at α glyceridic carbons and linoleic approximately 52% distributed at β glyceridic carbon.

KEY WORDS: Gated decoupled ^{13}C NMR, Acyl composition, Acyl positional distribution, Triacylglycerols, *Jatropha curcas* oil

INTRODUCTION

Carbon-13 NMR has been shown to be an adequate method for defining both the acyl distribution and positional placement on the glycerol backbone in triacylglycerols [1-3]. The advantages of NMR technique in achieving these goals over other techniques like high-performance liquid chromatography [4-5], supercritical fluid chromatography [6] and gas chromatography [7, 8] derive from the following facts. The NMR gives a spectrum where each nucleus is represented by a peak at a particular frequency. The area under the peak arising from each ^{13}C nucleus is proportional to the number of nuclei in that environment under certain experimental conditions. No elaborate sample preparation step is required and finally the NMR technique is a non destructive technique enabling the researcher to recover the sample for further analysis.

There are scanty reports on the fatty acid composition of under-utilised tropical seed oils in the literature. Some of these reports appear to show wide variation. We have therefore began a systematic study of these seed oils by ^{13}C NMR to enable us (i) to confirm the presence of the reported fatty acids, (ii) to identify and semi-quantitate the fatty acid present, and (iii) to determine the fatty acid distribution on the glycerol backbone. The present effort reports some structural and compositional information of the *Jatropha curcas* (an Euphorbiceae) seed oils that could be directly provided by ^{13}C NMR spectroscopy alone. The quantitative integrity of the NMR derived acyl distribution has been verified by gas chromatographic analysis of the oil.

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EXPERIMENTAL

Seeds of *Jatropha curcas* (JTC) were purchased from various markets in Ibadan, Akure and Ado-Ekiti, all in the South-Western part of Nigeria. The seeds were screened, washed and oven dried (103 ^{0}C). 30 g of the oven-dried samples were extracted in Sohxlet apparatus with chloroform-methanol mixture (2:1) for 20 h under nitrogen atmosphere. The extracts were dried under reduced pressure in a rotary evaporator. Toluene was added to ensure removal of any water through azeotropic distillation.

The extracted oil was purified. 2 g of extracted oil was percolated through a silica gel (15 g) column with a mixture of petroleum ether (b.p. 40-60 ^{0}C) and diethyl ether (95:5, v/v, 150 mL). The eluate was evaporated under reduced pressure to 5 mL portion and this portion further concentrated by a gentle blow of nitrogen gas to yield a mixture of triacylglycerols (1.72 g).

The ^{13}C NMR spectra of the samples were recorded on a Bruker AM-400 (Bruker Instruments, Inc., Karlsruhe, Germany) Fourier transform spectrometer operating at 100.6 MHz. Triplicate samples of the purified oil (300 mg) were dissolved in 1 mL of $CDCl_3$ and introduced into a 10 mm NMR tube. The gated decoupling pulse sequence was used with the following parameters: Number of scans 512, acquisition time 1.366 s, pulse width 10.3 s, delay time 1.0 s, free induction decay (FID) is transformed and zero filled to 300 k to give a digital resolution of 0.366 Hz/point.

Gas Chromatographic analyses of the oils were carried out by injecting 0.2 μL of their methyl carboxylates into a Hewlett-Packard 5890 GC (Hewlett-Packard Co; Palo Alto, Ca, USA). The column was HP Ultra Performance coated with crosslinked 5% phenol + 95% polysiloxane (30 x 0.25 nm, 0.2 μ coating thickness). Temperature programming was as follows: initial temperature, 160 ^{0}C for 2 min, temperature increased at 2.5 ^{0}C/min up to 300 ^{0}C and maintained at this final temperature for 5 min. Injector and detector temperatures were 280 ^{0}C and 340 ^{0}C, respectively.

RESULTS AND DISCUSSION

Table 1 presents the important signals in the ^{13}C NMR spectra of JTC oil. The ^{13}C NMR results are discussed based on the four important regions in the spectrum that provide essential information regarding the structure of the acyl groups and their distribution on the glycerol backbone. These regions are (i) C-1 carbon shift region, (ii) C-2 carbon shift region, (iii) the C-3, allylic, ω1-ω3 carbon shift regions, and (iv) olefinic carbon shift region. For convenience we abbreviate saturated acyl group as Sat, oleate, [18:1(9Z)] as O, and linoleate [18:2(9Z, 12Z)] as L.

C-1 Carbon shift region (ca 174 ppm). The high resolution ^{13}C spectrum of the carbonyl carbons showed three signals at 173.338, 173.302 and 172.887 ppm. Referring to established data [9-11], two of the signals can be paired (173.302/172.887) with a chemical shift difference value (Δδ) of 0.415. The highest chemical shift 173.338 in the spectra can be assigned to the carbonyl carbons of Sat in α position. Instead of relying solely on the chemical shift values, we have also made use of the chemical shift difference values to ascertain the type of the ester and their positions on the glycerol backbone throughout this discussion. The pair of signals 173.302/172.887 are therefore assigned to C-1 of O and L distributed in the 1,3 glyceridic, i.e.(α) and 2 glyceridic, i.e. (β) positions.

C-2 Carbon shift region (ca 34 ppm). Four signals 34.223, 34.114, 34.085 and 34.055 ppm appeared in this region. The signals 34.223 and 34.055 ppm can be paired (Δδ = 0.168 ppm).

These shifts are assigned to the C-2 carbon atoms of Sat in the β and α glyceridic positions. The 34.114 ppm and the 34.0846 ppm are assigned to C-2 of O and L in the α position. These assignments were based on established data [9-11].

Table 1. ^{13}C NMR chemical shifts of *Jatropha curcas* oil.

Chemical shift (ppm)	Assignment
173.388	C-1, Sat
173.302	C-1, O, L (α)
172.887	C-1, O, L (β)
34.223	C-2, Sat (β)
34.114	C-2, O (α)
34.085	C-2, L (α)
34.055	C-2, Sat (α)
24.978	C-3, Sat (α)
24.906	C-3, Sat (β)
24.896	C-3, L (α)
24.833	C-3, L (β)
27.255	C-11, O
27.233	C-14, L
27.204	C-8, O
26.942	C-8, L
25.655	C-11, L
31.961	ω3, Sat
31.939	ω3, O
31.764	ω3, L
22.731	ω2, Sat
22.716	ω2, O
22.607	ω2, L
130.244	C-13, L
130.048	C-10, O (β)
130.033	C-10, O (α)
130.004	C-9, L
129.735	C-9, O (α)
129.706	C-9, O (β)
128.106	C-10, L (β)
128.091	C-10, L (α)
127.924	C-12, L (α)
127.909	C-12, L (β)

C-3, Allylic, ω2 and ω3 carbon shift region. There are four signals in the C-3 region (*ca* 24 ppm), 24.978, 24.906, 24.869 and 24.833 ppm. The 24.869 and 24.833 ppm can be paired ($\Delta\delta$ = 00.036 ppm). Referring to established data [9-11] this pair of signals are assigned to C-3 of L distributed in the α and β glyceridic positions. The 24.978 ppm and the 24.906 ppm could be assigned to C-3 of Sat in α position and β position. Twelve signals appear in the region 20-27 ppm. Since no signal is found in the region *ca* 32 ppm, the presence of *trans* ethylenic systems in the seed oil can be ruled out.

Published reports [9-11] have shown that the allylic carbon atoms in the α or β positions, viz C-8 and C-11of O, C-8, C-11 and C-14 of L cannot be readily differentiated. Thus five signals are expected in the allylic region for *cis* and *cis*,*cis*-isomers. This is indeed the case in the allylic region between 25.655-27.255 ppm. The signal at 27.255 ppm is due to C-11 carbon atom of O, the 27.233 ppm is due to C-14 carbon atom of L, the 27. 2041 ppm is assigned to C-8 carbon atom of O, the 25.6549 ppm due to C-11 of L and the 26.942 ppm due to C-8 of L. The relative intensities of the allylic methylene protons are distinct and the signals profile and intensities could serve as fingerprint for identification of the oil.

Lie Ken Jie and Lam [11] have observed a deshielding order for the shifts of ω3 carbon nuclei as follows, Sat (31.976 ppm) > O (31.954 ppm) > L (31.567 ppm). This trend was also observed by the same authors for ω2 carbon nuclei. The spectra of JTC also shows this deshielding effect, so the 31.961 ppm and 31.939 ppm signals are assigned to the shift of the ω3 of Sat, the signal at 31.764 ppm assigned to ω3 of O and the signal at 31.561 ppm assigned to ω3 of L. In the ω2 region the signal at 22.731, 22.716 and 22.607 ppm are assigned to ω2 carbons of Sat, O and L, respectively.

Olefinic carbon shift region. There are ten signals in this region. Ng [3] observed that the chemical shift between a pair of peaks becomes smaller for the olefinic carbon nearer to the methyl end of the fatty acid chain, i.e. in the oleic chain, magnitude of peak separation is in the order C-9 > C-10 and in the linoleic chain the order is C-9 > C-10 > C12 > C-13. He also observed that in the oleic chain, the peak for C-9 attached at β glyceridic position appears at a lower frequency to that attached at the position and that the reverse order holds for C-10 [12]. These high/low frequency alternations in peak positions were also observed among the olefinic carbons of the linoleic chain. In general in the oleic chain Δδ between C-10 and C-9 α positions is 0.30 ppm and β positions is 0.34 ppm, while in the linoleic chain Δδ between C-13 and C-9, α positions is 0.20 and β positions is 0.24 ppm and Δδ between C-10 and C-12, α positions is 0.17 and β positions is 0.19 ppm. Based on the foregoing difference values and other established data [9-11, 13] the peaks in the olefinic region of JTC oil were assigned as presented in Table 1.

The intensities of the peaks in general show that O is slightly more abundant than L in JTC oil. The higher intensity peak in the pair for each olefinic carbon shows that more of O are attached to the α than to the β glyceridic position. The sharpness of the C-13 and C-9 of L clearly indicate that they are single peaks. However, their chemical shift difference (Δδ = 0.24 ppm) and the pair of peaks observed for C-10 and C-12 of L clearly point to the fact that more of the L are attached at the β than to the α glyceridic positions.

Semi-quantitative analysis of the fatty acid composition

Our results so far have revealed that JTC oil is composed of some Sat, O and L. For oils with non-complex composition like this, the peaks at *ca* 24 ppm belongs to C-3 of all the chains present so represent the total number of saturated, monoene and diene chains. The peaks at *ca* 25 ppm belongs to C-11 that is allylic to both double bonds of a cis-cis diene (linoleic) such that they represent the total total number of diene chains. The peaks at *ca* 27 ppm belong to the two carbons allylic to cis double bond, i.e C-8, C-11 of O and C-8, C-14 of L, such that they represent twice the total number of monoene O and diene L chains [14]. The areas under these peaks therefore permit quantitative analysis of the percentage of Sat, O and L. Integrals of these peaks at *ca* 24, 25, and 27 ppm are designated as a, b, and c, respectively, and the percentage composition of the oil is calculated as:

Percentage of Sat = [(a – 0.5c)/a] x 100

Percentage of L = (b/a) x 100

Percentage of O = [(0.5c- b)/a] x 100.

For the sample of JTC, a = 0.50, b = 0.16, and c = 0.73. The percentage of the fatty acids derived from NMR spectra is presented in Table 2 along side those obtained by GC analysis by us and some other workers. There is a very good agreement between our GC and NMR results for fatty acid composition. There is a fair agreement between our GC results and those of other workers. Specifically, NMR identified Sat, O and L as being present in JTC oil.

Table 2. Fatty acid composition of *Jatropha curcas* oil (%).

Fatty acid	a	b	c	d	NMR
Myristic	-	-	-	8.2	*
Palmitic	13.97	14.16	7.02	15.3	*
Palmitoleic	2.10	-	-	-	-
Stearic	16.42	7.68	13.02	6.8	*
Oleic	42.04	46.72	46.64	41.3	41
Linoleic	25.47	30.31	31.72	28.4	32
Arachidic	-	-	1.60	-	-
∑Saturated	30.39	21.84	20.04	30.3	27
∑Unsaturated	67.51	77.03	78.36	69.7	73

a- values reported by Mustafa *et al.* [15]. b- values reported by Nasir *et al.* [16]. c- values reported by Mehta and Gokhale [17]. d- values obtained in this present effort by gas chromatographic technique. NMR-values obtained in this present effort by ^{13}C NMR technique. *Quantitated together with other saturates.

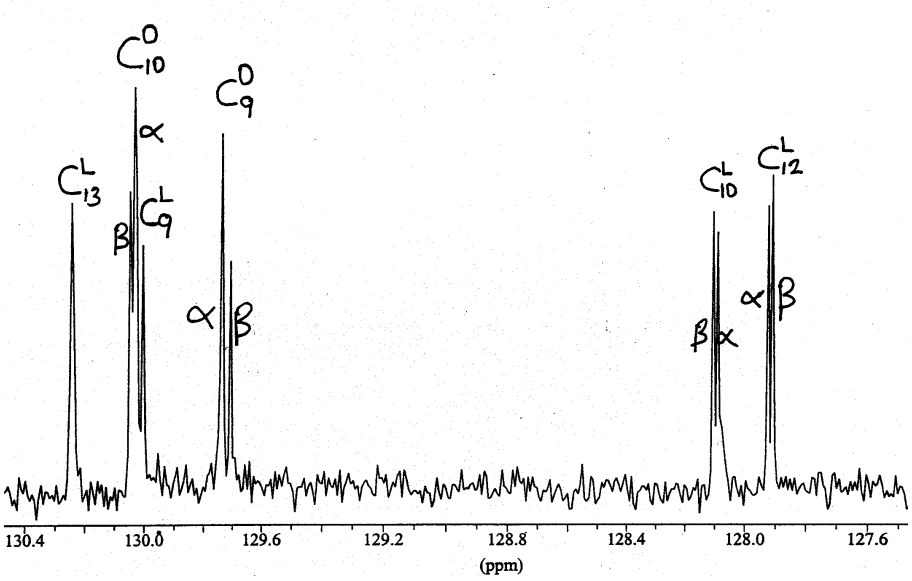

Figure 1. Proton decoupled ^{13}C NMR (100.6 MHz) of the olefinic carbons of the triacylglycerols of *Jatropha curcas* seeds oil. In the assignment of the peaks, the superscripts of symbol C are defined as follows, O for oleic and L for linoleic. The subscripts of symbol C represents the specified carbon in the fatty acid chain.

Acyl positional distribution

A high (or medium) field ^{13}C NMR spectrum can distinguish the olefinic carbons of the β-chain from those of the α-chain [3]. The assignment of the signals to the olefinic carbons of the fatty acid chain attached to the α or β glyceridic positions presented in Figure 1 were done as described by Ng [3]. Integration of the pair of peaks for C-9 of the oleic chain and of the pair of peaks for C-10 of the linoleic chain in a spectrum obtained under suitable condition of gated decoupling, permits quantitative analysis of the unsaturated chain attached to the α and β positions Tulloch [18]. Integration of the C-9 of the oleic and C-10 of the linoleic chain in Figure 1 revealed that 58% of oleic were attached to the α position of the glycerol backbone and that 52% of linoleic were attached to the β position of the glycerol backbone.

CONCLUSION

Our experimental results have further proved ^{13}C NMR spectroscopy to be a rapid and satisfactorily accurate tool for evaluation of acyl composition of oils with non complex composition. Based on the agreement between our NMR spectroscopic and gas chromatographic results on the acyl composition of JTC oil, we can reasonably state that the oil of JTC grown in the South-Western part of Nigeria contains saturated (*ca* 30.3%), oleic (*ca* 41.3%) and linoleic (*ca* 28.4%) acids. While the saturated acids are distributed randomly in the α and β glyceridic positions, the oleic acids are mostly attached at the α glyceridic position and the linoleic acids are mostly attached at the β glyceridic position. Specifically from the shifts of the olefinic carbons, about 58% of O is distributed in the α positions (see signal at 129.7 ppm) while L is distributed about 52% in the β position of the glycerol backbone (see signals at 127.9 and 128.0 ppm).

ACKNOWLEDGEMENT

Dr E.T. Akintayo is grateful to the Alexander von Humboldt Foundation of the Federal Republic of Germany.

REFERENCES

1. Wollenberg, K.F. *J. Am. Oil Chem. Soc.* **1990**, 67, 487.
2. Bergana, M.M.; Lee, T.W. *J. Am. Oil Chem. Soc.* **1996**, 73, 551.
3. Ng, S. *Lipids* **1984**, 19, 56.
4. Itabashi, Y.; Myher, J.J.; Kuksis, A. *J. Am. Oil Chem. Soc.* **1993**, 70, 1177.
5. Laakso, P.; Kallilo, H. *J. Am. Oil Chem. Soc.* **1993**, 70, 1161.
6. Lee, T.; Bobik, E.; Malone, W. *J. Assoc. Off. Anal. Chem.* **1991**, 74, 533.
7. Savage, P.; McNeil, D.L.; Dutta, P.C. *J. Am. Oil. Chem. Soc.* **1997**, 74, 755.
8. Parcerisa J.; Boatella, J.; Codony, R.; Farran, A.; Garcia, J.; Lopez, A.; Rafecas, M.; Romero, *Food Chem.* **1993**, 48, 411.
9. Lie Ken Jie, M.S.F.; Lam, C.C.; Bonnie, F.Y.Y. *J. Chem. Res.* **1992**, (S) 12-13, (M) 0250-0272.
10. Gunstone, F.D.; *High Resolution ^{13}C NMR Spectroscopy of Lipids* in *Advances in Lipid Methodology-Two*, Christie, W.W. (Ed.); The Oily Press: Dundee; **1993**; pp 1-68.
11. Lie Ken Jie, M.S.F.; C.C., Lam *Chem. Phys. Lipids* **1995**, 78, 1.

12. Ng, S. *J. Chem. Soc. Chem. Commun.* **1983**, 179.
13. Chapman, D.; Goni, F.M.; Gunstone, F.D. *Physical Properties, Optical and Spectra Characteristics* in *Lipid Handbook*, Gunstone, F.D.; Harand, J.L.; Palley, F.B (Eds.); University Press: Cambridge (U.K.); **1994**; pp 487-557.
14. Ng, S.; Ng, W.L. *J. Am. Oil Chem. Soc.* **1984**, 60, 1266.
15. Mustafa, A.I.; Hussain, M.A.; Amin, M.N.; Chowdhury, G.N.I. *J. Bangladesh Chem. Soc.* **1997**, 10, 39.
16. Nasir, M.K.A.; Memon, G.M.; Valhari, M.U.; Khatri, L.M. *Pak. J. Sci. Ind. Res.* **1988**, 31, 566.
17. Mehta, T.N.; Gokhale, M.W. *Indian J. Appl. Chem.* **1964**, 27, 109.
18. Tulloch, A.P. *Lipids* **1982**, 17, 544.