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FATTY ACID DISTRIBUTION IN TRIACYLGLYCEROLS FROM ARIL AND COTYLEDON OILS OF *AFZELIA CUANZENSIS**

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Key Word Index—*Afzelia cuanzensis*; Caesalpinaceae; cotyledons; arils; oil composition; ^{13}C NMR; acyl-chain distribution.

Abstract—The seeds of *Afzelia cuanzensis* were previously reported to have a high oil content, the aril oil being made up of the common C_{18} saturated, mono- and di-unsaturated fatty acids, whilst the oil from cotyledons, beside oleic and linoleic acids, contained large amounts of crepenynic and dehydrocrepenynic acids (together 60%). We determined the acyl composition and acyl positional-distribution (1,3-acyl and 2-acyl) of triacylglycerols from arils and cotyledons, respectively, by ^{13}C NMR spectroscopy. Positional analysis of the oil from cotyledons indicated a preferential placement of oleic and linoleic (both 65%) than crepenynic and dehydrocrepenynic acids (both 25%) at position 2. In both aril and cotyledon oils, the saturated acids are exclusively in the 1,3-position of glycerol.

INTRODUCTION

The genus *Afzelia* comprises 14 species, eight of them being in Africa. The size of the tough ovoid fruits varies between 12 and 15×6–8 cm in axial and equatorial diameters, respectively, each fruit being six to 10 seeded [1,2]. The seed appearance is striking being made up of the deep-reddish aril and the pitch-black seed coat. There are no records that the seeds have been used by the indigenous population as a food source or for preparing beverages, in medicine or for other purposes.

It was apparent from pioneering studies reported by Pieraert and L'Heureux [3] that the oil of *Afzelia* seeds was made up of common C_{18} fatty acids (**1–3**) and of unusual fatty acids of equivalent chain length C_{20} and C_{22}. It was, however, Gunstone *et al.* [4] who identified the two major acids as crepenynic (**4**) and dehydrocrepenynic (**5**), leaving undefined the geometry of the C_{14}-C_{15} double bond in the latter component. Since this study, further information has been provided on the lipids present on the surface of the pods [5] and on the fatty acid composition of the oils from arils and coteyledons, respectively [6].

The present paper reports a ^{13}C NMR study from which the acyl chain composition of triacylglycerols and acyl chain positional distribution on the three positions of the glycerol moiety of the oils extracted from different parts of mature fruits of *A. cuazensis* were precisely determined.

(1) $CH_3(CH_2)_nCOOH$ $\quad n = 14, 16, 20$

(2) $CH_3(CH_2)_7-CH=CH-(CH_2)_7COOH$

(3) $CH_3(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7COOH$

(4) $CH_3(CH_2)_4-C\equiv C-CH_2-CH=CH-(CH_2)_7COOH$

(5) $CH_3(CH_2)_2-CH=CH-C\equiv C-CH_2-CH=CH-(CH_2)_7COOH$

RESULTS AND DISCUSSION

Aril and cotyledon tissues were almost completely devoid of water and the proportion of oil in arils and cotyledons were *ca* 30% and 15% by weight, respectively. Since the cotyledon oil is sensitive to air and to light, the NMR spectra were registered immediately after oil extraction. ^1H NMR spectra of *Afzelia* oils did not reveal relevant information on the structure of the triacylglycerol acyl-chains; this is due to the general failure of the method in separating long-chain proton signals. The complexity was even worse in *Afzelia* seed oils where several different acyl chains are present.

Data available in the literature [7], and from our own systematic study on acylglycerols of vegetable oils, have demonstrated that high resolution ^{13}C NMR spectroscopy and, in particular, the spectral regions relative to carbonyl and olefinic carbon signals, enable one to discern between saturated and unsaturated homologous acyl chains and also, to determine their position of esterification on the glycerol backbone. ^{13}C NMR spectra were run adopting appropriate acquisition and processing parameters in order to obtain spectral

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resolution of 0.2 Hz/pt for all the chains, along with their 1,3 and 2 splittings resolved to such an extent as to enable accurate integration of signals. ^{13}C NMR spectra of both aril and cotyledon oils exhibited signals for C-2 and C-1,3 of the glycerol moiety in acylglycerols at δ 68.86 and 62.07, respectively [8]. However, in the glycerol region no signals for mono- and diacylglycerols were detected.

The C-1 ^{13}C NMR spectral region of fatty acids covers a very narrow chemical shift-range with overlapping of several resonances. Nevertheless, when using NMR as analytical tool, the carbonyl region remains the only way of calculating the saturated chain content of triacylglycerol mixtures in vegetable oils. Most of the signals for carbonyl carbons C-1 were readily assigned to the different chains by comparison with chemical shifts of standard triacylglycerols. The C-1 signals of the various chains at the 1,3- and 2-position of glycerol were assigned on the basis of the relative signal sizes in a 2:1 ratio, since in homogeneous acylglycerols there are two chains at the 1,3- and one in the 2-position. Thus, the signals of saturated (δ 173.24), oleic (δ 173.20) and linoleic (δ 173.19) chains esterified at the 1,3-position of glycerol were identified. The oleic (δ 172.79) and linoleic (δ 172.78) chains esterified at the 2-position were shifted downfield by *ca* 0.41 ppm relative to the same chains at the 1,3-position. Peaks for saturated chains which were found only in the 1,3-position of glycerol, could not be resolved into their homologues.

In the ^{13}C NMR spectrum of the cotyledon oil, two peaks at δ 173.17 and 172.77 were clearly distinguishable from those of the commonly encountered fatty acid chains (Table 1). In agreement with the shift-trend found for the model chains described above, the two resonances (1,3-diacyl and 2-acyl) were separated by *ca* 0.40 ppm. This observation, and in consideration of previous literature data, suggested that the two peaks were the overlapping resonances of compounds **4** and **5** at the 1,3- and 2-position of glycerol, respectively [4, 6].

The complete qualitative and quantitative picture of the oil sample compositions was achieved by analysing the resonances of unsaturated carbons of multiple-bond systems present in the acylglycerols making up the two oil samples. The signals of double bond carbons of oleyl and linoleyl chains at the 1,3- and 2-positions of glycerol backbone were detected and found to be in agreement with literature data and accordingly assigned [9]. The observed and precisely recorded shift separation of the double bond carbons of oleic and linoleic chains agree well with the values calculated by the recently proposed σ-inductive theory, by which the chemical shifts of unsaturated carbons in linear long-chain esters and acids can be predicted. The theory quantitates the transmission of the dipolar effect of the carbonyl group upon multiple bonds in monoenes and of a multiple bond upon another multiple bond in non-conjugated polyenes [10, 11].

A notable spectroscopic feature in the present study was that the unsaturated carbons of the oleic and linoleic chains resonate as doublets, whose splittings were found to be dependent on the 1,3- or 2-position of glycerol. The assignments of 1,3- and 2-splitting of unsaturated carbon signals were confirmed by running ^{13}C NMR spectra of homogeneous triglycerides, such as triolein and trilinolein. It was observed that the splittings for unsaturated carbons were as follows. For acyl chains at the 2-position of glycerol, the signal of the double bond carbon closer to C-1 was shifted upfield compared with that of 1,3-chains. The opposite was observed for the double bond carbon furthest away from C-1. Thus, the recorded difference between the C-9 signals of the oleyl and linoleyl chains at the 1,3- and 2-position of glycerol was 0.03 ppm, whilst those for C-10 resonances were markedly lower and opposite in sign, -0.01 and -0.02 ppm for oleyl and linoleyl chains, respectively. The linoleyl C-12 was split by 0.01 ppm, whilst no splitting was detected for C-13. The above detailed spectroscopic features proved to be a powerful diagnostic tool of general application for determining the acyl chain composition and the acyl chain positional-specificity to the glycerol moiety in vegetable oils made up of monounsaturated and polyunsaturated non-conjugated fatty acids [7].

The observed double-triple and double-triple-double unsaturated carbon signal systems of crepenynic and dehydrocrepenynic chains, respectively, provided the expected spectral evidence proving the presence of the two acids at all the glycerol positions [12, 13]. The C-9 resonances of **4** and **5** gave splitting values of 0.03 ppm identical to those found for oleyl and linoleyl chains, while C-10 exhibited a shift of -0.02 ppm between the 1,3- and 2-positions of the same sign, being, however, higher than those found for oleyl and linoleyl chains. For C-12 in chain **4**, a 0.02 ppm splitting value was detected.

Methyl and methylene envelope signals were of further interest for oil analysis. The C-2 of all the chains resonated as a single peak split according to the chain position on glycerol, C-2 at the 2-position of glycerol having the higher chemical shift. The same was observed for C-3. For C-2 and C-3, the observed differences between the resonances of 2- and 1,3-chains were 0.17 and 0.05 ppm, respectively, as previously reported for common C_{18} fatty chains [9]. The methylene carbons C-4 to C-7 and C-12 to C-18 in compounds **1–3** were assigned according to both peak integration and measurements of ^{13}C NMR T_1-relaxation times, which were found to increase regularly from the glycerol backbone along the fatty acid chains [14].

The allylic carbons C-8 of crepenynic and dehydrocrepenynic chains at δ 27.03 and 27.09, respectively, were shifted upfield from C-8 of oleic (δ 27.14) and linoleic chains (δ 27.16), all the shifts confirming the *cis*-configuration of the C9-C10 double bonds [12]. The C-11 signals, the methylene between the double and triple bonds of **4** and **5**, were widely resolved and shifted upfield from the methylene C-11 of oleyl and linoleyl chains, which resonate at δ 27.19 and 25.60,

Table 1. ^{13}C NMR chemical shifts for **4**, **5**, oleic (18:1), linoleic (18:2) and saturated (n:0) chains at the 1,3- and 2-glycerol positions (75 MHz, $CDCl_3$, TMS as int. standard)

C	**1** n*:0	**2** 18:1 9c		**3** 18:2 9,12c		**4** 18:2 9c12a		**5** 18:3 9c12a14c	
	1,3-Pos.	1,3-Pos.	2-Pos.	1,3-Pos.	2-Pos.	1,3-Pos.	2-Pos.	1,3-Pos.	2-Pos.
1	173.24	173.20	172.79	173.19	172.78	173.17	172.77	173.17	172.77
2	34.02	33.98	34.15	33.98	34.15	33.98	34.15	33.98	34.15
3	24.79	24.79	24.84	24.79	24.84	24.79	24.84	24.79	24.84
4	29.11†	29.06	29.02	29.06	29.02	29.03†		29.03†	
5	29.25	29.15	29.17	29.15	29.17	29.03†		29.03†	
6	29.46	29.09†		29.09†		29.03†		29.03†	
7	29.64	29.69†	29.68†	29.58	29.60	29.10†		29.10†	
8	29.68	27.14		27.16		27.03		27.09	
9	29.68	129.67	129.64	129.96	129.93	131.08	131.05	131.54	131.51
10	29.68	129.97	129.98	128.03	128.05	125.10	125.13	124.33	124.35
11	29.68	27.19		25.60		17.15		17.93	
12	29.68	29.74		127.86	127.85	78.30	78.28	92.32	
13	29.68	29.30		130.18		80.03		77.22	
14	29.68	29.50		27.17		18.73		142.62	
15	29.34	29.29		29.32		28.73		109.33	
16	31.90	31.88		31.50		31.06		32.04	
17	22.66	22.66		22.54		22.18		22.12	
18	14.09	14.09		14.04		13.97		13.72	

*n = 16–22. Data for C_{18} chain.

†Tentative assignment.

respectively (Table 1). They were assigned from ^{13}C–^{1}H correlations. The H-11 protons of compounds **4** and **5** resonated as broad peaks, whose coupling constants remained undefined, at δ 2.88 and 3.07, respectively [13]. C-14 and C-15 of **4** did not exhibit 1,3- and 2-position splitting, resonating as single peaks and were assigned according to literature data [12].

C-16 to C-18 in all the compounds examined gave well-resolved peaks. The signals were assigned on the basis of the additive rule for ^{13}C-chemical shifts of alkanes and of the peak integrals, which were predicted from the relative chain composition deduced from carbonyl and olefinic regions [15]. The shift at δ 32.04 for C-16 in compound **5**, did not give evidence of the configuration of the C-14 double bond. In fact, C-16 is allylic to the C_{14}-C_{15} double bond, but its chemical shift is also influenced by the chain-end methyl group. Thus, the definitive evidence for the presence of the *cis*-configuration of the C_{14}-C_{15} double bond is given by the ^{1}H NMR spectrum. The double triplet at δ 5.82 (J = 10.4, 7.1 Hz) was due to the olefinic proton of the C-15 double bond in conjugation with a triple bond. The configuration of the C-14 double bond was assigned as *cis* by the proton coupling constant J = 10 Hz of the hydrogen atoms at C-14 and C-15.

The chemical shifts of the acylglycerol models illustrated above represented the basis for determining the composition and positional specificity of acyl chains of the oils from *A. cuanzensis* (Table 2). The acyl chain composition for aril oil was calculated on the basis of the integrated areas of the C-1 signals of all the chains at 1,3- and 2-position of glycerol, respectively. In cotyledon oil from *Afzelia*, where C-1 of **4** and **5** chains were not resolved, acid concentrations and positional specificity were obtained from the olefinic region, in which all the carbons were baseline-resolved, thus enabling accurate integration of signals. The compositional data obtained from unsaturated carbons of **4** and **5** moieties were adjusted for saturated chain data derived from C-1 signals. The 2-proportion of an acid, which gives the positional specificity of that chain for the 2-position of glycerol, was derived from the area ratios of the same chain at the 1,3- and 2-positions, respectively. The results obtained, expressed in % molar fractions, are reported and compared in Table 2, where GC composition is also shown [6].

In conclusion, while there is a great deal of data in the literature on the oil compositions of many plant species, to our knowledge, the occurrence of such chemically different oils in two parts of the same seed is a distinctive feature of *A. cuanzensis*, and possibly of other *Afzelia* species. The aril oil triaglyglycerols were esterified with oleyl, linoleyl and saturated chains, the latter being the major chains present (43.3%). The cotyledon oil, whilst having the common fatty chains found in the corresponding aril oil, was characterized by the polyunsaturated chains of **4** and **5**, acid **4** being the major component (45.7%), followed by dehydrocrepenynic acid **5** (17.2%).

The data given in Table 2 show that saturated chains were found only at the 1,3-positions of glycerol in both aril and cotyledon oils, whereas oleyl and linoleyl chains were preferentially acylated at the 2-position. These findings are in agreement with the well-known biosynthetic general rule [16], that in vegetable oils, the 2-position of glycerol is acylated almost exclusively by unsaturated C_{18} chains. It is of interest to note that, in *Afzelia*, oleic and linoleic occupy the glycerol 2-position at 65–67% of their total content, being far from the 33.3% value for a purely random distribution. On the other hand, acid **4** and **5** are found on the same position in the range 25–28%, fairly close to a random esterification pattern. The overall fatty acid NMR compositional data of aril and cotyledon oils were in fair agreement with GC data [6]. However, GC data for **2** and **4** were markedly different from the values found in the present work. Although crepenynic and dehydrocrepenynic acids have been identified in hydrolysates of *Afzelia* seed oils [1–6] and glycerol tridehydrocrepenynate has been found to be the major triacylglycerol of the Basidiomicete, *Craterellus cornucopioides* [13], this is the first report describing the positional distribution of **4** and **5** in mixed triacylglycerols.

EXPERIMENTAL

Afzelia cuanzensis fruits originated from areas around Maputo (Mozambique). Seeds with aril, stored at −10°, withdrawn as required and analysed, showed no changes in their triacylglycerol composition even after long periods of time. Two seeds (7 g) were dissected into black cotyledons (6 g) and reddish arils (1 g). Freshly mechanically drilled arils were extracted

Table 2. Fatty acid composition of different parts of *Afzelia cuanzensis* fruits by ^{13}C NMR (75 MHz, $CDCl_3$, TMS as int. standard)

	Aril oil		Cotyledon oil	
Fatty acid	Composition*	2-Proportion	Composition	2-Proportion
1, (16–22):0	43.3 (42)	0	10.8 (10)	0
2, 18:1 9c	33.9 (27)	51.7	6.5 (19)	66.7
3, 18:2 9,12c	22.8 (27)	61.5	19.8 (22)	65.2
4, 18:2 9c12a	0	0	45.7 (30)	28.3
5, 18:3 9c12a14c	0	0	17.2 (14)	25.0
18:3 9,12,15c	0 (2)	—	0 (2)	—

*GC composition in parentheses [6].

in a Soxhlet with petrol (bp 40–70°) for 3 hr. In a similar way, the finely pulverized seed cotyledons were extracted. The solvent was evapd off under a stream of N_2 to give almost pure oil samples, which were analysed by ^{13}C spectroscopy. Spectra were recorded at 75 MHz in $CDCl_3$ using TMS as int. standard.

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