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SEED OIL ALKANES FROM LEGUMINOSAE SPECIES: EVEN-CARBON NUMBER PREFERENCE

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Key Word Index—Caesalpinoideae, Mimosoideae, Papilionoideae, Leguminosae; seed oil alkanes; *n*-alkanes; even-carbon number alkanes.

Abstract—GC and GC-MS have been used to study the distribution of *n*-alkanes in the seed oil from 60 samples of 43 Leguminosae species. The occurrence of high percentages of low M_r, even-carbon numbered alkanes was the most important feature in relation to earlier reports on plant hydrocarbons. The main seed oil alkane in almost all species investigated was hexadecane followed by octadecane, eicosane and tetradecane in decreasing order. All samples studied from the three subfamilies presented almost the same pattern with little quantitative and qualitative differences. The presence of this distinctive carbon range of aliphatic paraffins suggests that the inner conditions could lead to a change in chain-length specificity and/or activity of some enzyme systems. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The long, straight chain hydrocarbons occurring in higher plants have been studied extensively [1–4]. The alkane distribution patterns have also been determined in species growing in different environmental conditions [5–7] and, in some cases, this fractions has been used as a taxonomic aid [8–10]. These studies relate mainly to hydrocarbons from cuticular waxes.

Seed oil and internal hydrocarbons include a small but significant proportion of alkanes, which may participate in maintaining inner cell structure and may act as energy storage components. However, few investigations of these components have been reported [11, 12].

Although a wide range of alkane patterns differing in their M_r distribution has been found in living organisms, odd-carbon numbered *n*-alkanes are the major constituents in higher plants. The biosynthesis of such components has been extensively investigated [13–20].

All the cited cases, which report no alternation of odd and even-carbon number alkanes or flatter distribution with an increase in the relative proportion of the even-carbon number members of the series, were obtained from prolonged extraction of leaf waxes or other tissues or from macerated plant material. In those cases the intracellular lipids would have been included in the lipid fractions examined [21–23].

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At present, there are no studies of the Leguminosae or related families, where the even-carbon numbered alkanes from seed lipids are dominant.

The findings presented here are concerned with the unusual composition of the seed oil alkanes of 43 species belonging to the three subfamilies of the Leguminosae, as revealed by the application of IR, GC and GC-MS techniques.

RESULTS AND DISCUSSION

The alkane composition of the seed oils of the species studies (Table 1), is presented in Table 2. Nineteen *n*-alkanes were identified. IR spectra confirmed their identity as paraffins (2825, 2854, 1481 and 1375 cm^{-1}), and the lack of absorption at 1720–1740 cm^{-1}, 3600–3400 cm^{-1} or 1100–1250 cm^{-1} confirmed the absence of carbonyl, hydroxy or internal oxygen-containing components.

The mass spectra of the *n*-alkanes showed for each $[M]^+$ at m/z 198, 209, 226, 239, 254, 267, 281, 295, 309, 323, 337, 351, 365, 379, 393, 407, 421, 435 and 449 which correspond to C_{14}–C_{32} respectively. There was no evidence of branched or unsaturated components.

Qualitative and quantitative variations were observed among the species. The main features of the alkane distribution patterns were as follows: (i) The even-numbered compounds exceeded their odd-carbon neighbours (62.8–99.2% of total *n*-alkanes). (ii)

Table 1. Collection data (Caesalpinoideae, Mimosoideae and Papilionoideae Subfamilies)

Taxon	Voucher No	Population site
Caesalpinoideae Subfamily		
Caesalpinieae Tribe		
Caesalpinia gilliesii Wall. ex Hook (1)	RHF 5030 (BAB)	
Caesalpinia gilliesii (2)	BAB 91963	Córdoba: Capital
Caesalpinia gilliesii (3)	BAB 91953	Córdoba: Mina Clavero
Cercidium praecox (R. et P) Harms subsp. *praecox*	RHF 5036 (BAB)	Santiago del Estero: 20 km from Sumampa
Cassieae Tribe		
Senna alata L.	CTES 29436	Corrientes: Capital
Chamaecrista venturiana Irwin et Barneby	RHF 5377 (BAB)	Bolivia
Cercideae Tribe		
Bauhinia forficata Link subsp. *pruinosa* (Benth.) Fortunato et Wunderlin.	BAB 91931	Córdoba: Capital
Mimosoideae Subfamily		
Mimoseae Tribe		
Prosopis alba Griseb. (1)	18'88	Santiago del Estero:
Prosopis alba (2)	532'90	Lat. 28°57' Long. 66°57'
Prosopis nigra (Griseb.) Hier. (1)	62'88	Lat. 25°05' Long. 64°09'
Prosopis nigra (2)	68'88	Salta
Prosopis ferox Griseb.	76'89	Salta: Bosque de Tintin
Prosopis caldenia Burkart	542'90	Lat. 32°20' Long. 65°10'
Prosopis kuntzei Harms	k09	Loreto: Río Namby
Prosopis flexuosa DC.	575'91	La Rioja: Tinogasta
Prosopis argentina Burkart	38'88	Mendoza: Tucunucu
Anadenanthera macrocarpa Humb. et Bonpl. ex Willd.	BAB 91929	Salta: Capital
Anadenanthera colubrina (Vell.) Brenam	CTES 29437	Corrientes :Capital
Parapiptadenia excelsa (Griseb.) Burkart f. *jujuyensis* Burkart	BAB 91927	Salta: Capital
Desmanthus virgatus (L.) Willd.	BAB 91962	Córdoba: Saldán
Acacieae Tribe		
Acacia caven (Mol.) Mol. var. caven (1)	CORD 186	Córdoba: La Calera
Acacia caven (2)	BAB 91947	Córdoba: Villa Allende
Acacia aroma Gill. ex Hook. (1)	CORD 188	Córdoba: Route 28, km 873
Acacia aroma (2)	CORD 191	San Juan: Route 12, km 87
Acacia aroma (3)	CORD 196	San Juan: Route 12, km 66
Acacia aroma (4)	BAB 91936	Córdoba: Villa Allende
Acacia aroma (5)	BAB 91948	Córdoba: Villa Allende
Acacia furcatispina Burkart (1)	CORD 187	San Juan: Route 12, km 87
Acacia furcatispina (2)	BAB 91930	Córdoba: El Diquecito
Acacia atramentaria Benth. (1)	BAB 91932	Córdoba: Capital
Acacia atramentaria (2)	BAB 91930	Córdoba: Route 38, km 49
Acacia parviceps (Speg.) Burkart	BAB 91928	Salta: Capital
Acacia horrida (L.) Willd.	BAB 91946	Chile: Route 5, km 80
Ingeae Tribe		
Inga uruguensis Hook. et Arn.	CTES 29439	Chaco: Río Paraná
Enterolobium contortisiliquum (Vell.) Morong	BAB 91925	Salta: Rosario de Lerma
Papilionoideae Subfamily		
Dalbergieae Tribe		
Tipuana tipu (Benth.) Kuntze	BAB 91954	Córdoba: Capital
Robinieae Tribe		
Sesbania punicea (Cav.) Benth	BAB 91933	Córdoba: Capital
Indigoferae Tribe		
Indigofera parodiana Burkart	RHF 5338 (BAB)	Córdoba: Río Tercero
Indigofera suffruticosa Mill.	RHF 5391 (BAB)	Bolivia: 5 km from Coroico
Desmodieae Tribe		
Desmodium cuneatum Hook. et Arn.	RHF 5341 (BAB)	Córdoba: Villa La Bolsa

Table 1—*continued.*

Taxon	Voucher No	Population site
Desmodium tortuosum (Sw) DC	RHF 5334 (BAB)	Córdoba : Parador El Condor
Desmodium neomexicanum Gray	BAB 91951	Córdoba : Mina Clavero
Phaseoleae Tribe		
Collaea speciosa (Lois.) DC.	RHF 5373 (BAB)	Bolivia
Galactia latisilicua Desv. (1)	BAB 91934	Córdoba : Capital
Galactia latisilicua (2)	BAB 91956	Córdoba : Carlos Paz
Galactia latisilicua (3)	BAB 91971	Córdoba : Villa Los Aromos
Cologania ovalifolia H.B.K.	BAB 91920	Salta : Route to Corralito
Centrosema virginianum (L.) Benth. (1)	BAB 91924	Salta : Campo Quijano
Centrosema virginianum (2)	BAB 91973	Córdoba : Agua de Oro
Rhynchosia edulis Griseb. (1)	BAB 91966	Córdoba : Cabalango
Rhynchosia edulis (2)	BAB 91970	Córdoba : Villa Los Aromos
Rhynchosia edulis (3)	BAB 91955	Córdoba : Capital
Rhynchosia edulis (4)	BAB 91974	Córdoba : Agua de Oro
Rhynchosia senna Hook. et. Arn.	BAB 91965	Córdoba : Mina Clavero
Aeschynomeneae Tribe		
Aeschynomene rudis Benth.	RHF 5375 (BAB)	Bolivia : Coroico
Stylosanthes montevidensis Vogel.	RHF 5332 (BAB)	Córdoba : Altas Cumbres
Stylosanthes gracilis H.B.K.	RHF 5336 (BAB)	Córdoba : Río Tercero
Adesmieae Tribe		
Adesmia volckmanni Phil.	RHF 5215 (BAB)	Neuquén : 38 km from Junín de los Andes. Route 40
Galegeae Tribe		
Glycyrrhiza astragalina Gill. ex Hook. et Arn.	RHF 5213 (BAB)	Neuquén : Junín de los Andes
Vicieae Tribe		
Lathyrus magellanicus Lam. *var. glaucescens* Speg.	RHF 4889 (BAB)	Tierra del Fuego

n-Hexadecane was the principal alkane in all species except in *Cologania ovalifolia* where *n*-octadecane was the dominant component. (iii) *n*-Octadecane, *n*-eicosane and *n*-tetradecane were present in significant amounts. (iv) Some species of *Acacia* (*A. aroma*, *A. atramentaria* and *A. parviceps*) presented a flatter distribution, by way of an increase in the odd-carbon numbered and long chain components. This feature was also encountered in *Sesbania punicea.* (v) The genus *Rhynchosia* (Phaseoleae tribe) was characterized by the presence of significant levels of dotriacontane (4.5–7.2%), an alkane not found in the other groups.

No variation was found in the alkane patterns of the same species originating from different localities. This feature could demonstrate the predominance of genetic factors over external conditions in these species.

Earlier investigation on the unsaponifiable matter from some species of this plant family found a mixture of C_{16}–C_{37} *n*-alkanes, with the ratio of odd to even number alkanes much less than that found in other higher plants [11]. Our results are in agreement with these findings, but the ratios found by us were much lower.

It has been postulated that two pathways must operate in the biosynthesis of hydrocarbons. As well as the constitution of epicuticular waxes varying between species [8–10, 23], as a result of plant age [24], and of the environmental conditions in which plants grow or to which they are exposed [1, 5, 7, 25], the inner tissue conditions could lead to a change in chain-length specificity and/or activity of some enzyme systems. Our results suggest that the preponderance of even and short carbon numbered alkanes in the seed oils of Leguminosae could be strongly tissue dependent, apparently as a result of tissue-specific differences in alkane biosynthesis.

Finally, the presence of this class of alkanes in Leguminosae seed oils suggests that this plant material should provide a good source for studying short chain alkane biosynthesis.

EXPERIMENTAL

Plant material

Details of plant material are given in Table 1. Voucher specimens are retained at the Herbarium of the Instituto de Recursos Biológicos, INTA, Castelar (BAB), at the Instituto de Botánica del Nordeste, Corrientes (CTES), at the Harbarium of the Museo Botánico (Facultad de Ciencias Exactas, Físicas y Naturales of the Universidad Nacional de Córdoba)

Table 2. Alkane composition (% total) of seed oils from Caesalpinoideae, Mimosoideae and Papilionoideae (Leguminosae) samples

Taxon	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32
Caesalpinoideae																			
Caesalpinia gilliesii (1)	10.3	tr	30.8	tr	24.4	5.0	14.9	tr	10.9	tr	3.2	tr	tr	tr	tr	tr	tr	tr	—
Caesalpinia gilliesii (2)	10.5	tr	30.6	tr	24.2	4.9	14.7	tr	11.1	tr	3.2	tr	tr	tr	tr	tr	tr	tr	—
Caesalpinia gilliesii (3)	10.5	tr	31.0	tr	24.7	4.7	15.1	tr	10.6	tr	3.2	tr	tr	tr	tr	tr	tr	tr	—
Cercidium praecox	8.4	1.9	26.2	2.2	20.3	3.5	11.6	tr	3.3	tr	3.5	2.1	5.5	9.2	tr	1.8	tr	tr	—
Senna alata	12.8	tr	26.2	tr	23.1	tr	11.8	tr	10.5	tr	6.8	tr	3.0	tr	5.0	tr	tr	tr	—
Chamaecrista venturiana	11.4	tr	30.5	tr	21.9	tr	11.9	tr	7.4	tr	6.5	3.0	3.8	1.7	0.9	0.6	tr	tr	—
Bauhinia forficata	16.2	tr	22.9	tr	14.4	tr	7.7	tr	6.0	tr	4.8	5.0	4.0	4.1	8.4	6.3	tr	tr	—
Mimosoideae																			
Prosopis alba (1)	12.0	0.6	28.1	tr	18.7	2.3	10.7	1.7	8.5	tr	3.0	tr	3.3	1.6	4.6	3.1	1.5	tr	—
Prosopis alba (2)	12.7	0.5	30.4	0.9	21.5	0.6	8.1	0.5	7.3	tr	3.6	tr	3.2	2.2	1.5	4.6	2.1	tr	—
Prosopis nigra (1)	11.7	1.3	32.8	1.0	24.1	0.8	8.6	0.9	8.7	tr	3.7	tr	2.8	tr	0.9	0.9	1.1	tr	—
Prosopis nigra (2)	18.7	1.0	28.7	0.8	18.5	0.4	11.8	1.2	11.1	0.4	3.1	0.4	1.6	0.8	0.4	0.6	0.4	tr	—
Prosopis ferox	5.4	0.3	27.2	0.3	26.2	0.1	16.5	tr	9.6	tr	5.9	tr	4.0	1.3	1.8	1.1	tr	tr	—
Prosopis caldenia	13.0	1.4	33.2	0.9	21.4	1.3	12.3	0.9	6.5	tr	3.4	tr	1.8	1.3	1.1	1.2	tr	tr	—
Prosopis kuntzei	6.9	1.2	26.4	1.6	23.3	1.5	13.8	1.2	7.9	1.6	4.6	1.0	2.8	1.0	4.4	0.6	tr	tr	—
Prosopis flexuosa	10.9	1.5	23.9	1.9	20.1	1.2	12.1	1.1	6.3	0.1	3.5	1.5	2.6	7.3	2.7	2.6	0.4	tr	—
Prosopis argentina	10.0	1.0	27.8	1.3	23.0	0.9	15.2	0.6	8.9	tr	5.0	tr	2.7	tr	1.6	tr	1.6	tr	—
Anadenanthera macrocarpa	10.8	3.6	19.2	1.9	19.1	6.7	12.4	1.6	6.5	1.4	4.1	1.6	2.1	2.6	2.7	1.7	1.9	tr	—
Anadenanthera colubrina	10.8	4.0	29.2	5.1	21.1	3.5	10.8	1.6	6.2	tr	3.4	tr	2.1	0.5	1.3	tr	0.1	tr	—
Parapiptadenia excelsa	6.9	1.6	19.1	2.3	14.1	6.3	8.6	2.1	3.9	1.6	2.0	6.2	5.6	6.1	3.9	5.3	4.3	tr	—
Desmanthus virgatus	11.8	1.5	28.9	1.8	21.9	1.3	13.3	1.1	7.9	tr	4.9	tr	2.7	0.4	1.5	0.1	0.7	tr	—
Acacia caven (1)	10.6	1.9	28.5	1.5	23.1	1.3	12.9	1.3	7.3	0.4	3.5	0.2	2.0	1.5	1.4	1.9	0.6	tr	—
Acacia caven (2)	10.9	1.8	28.7	1.5	22.8	1.3	12.4	1.3	7.5	0.4	3.8	0.2	2.2	1.5	1.3	1.6	0.7	tr	—
Acacia aroma (1)	8.1	1.5	18.3	3.9	12.8	3.6	7.8	0.3	4.9	0.2	3.2	1.2	3.0	6.7	10.8	9.8	3.8	tr	—
Acacia aroma (2)	8.2	1.5	18.3	3.8	12.9	3.6	7.9	0.3	4.9	0.2	3.2	1.2	3.1	6.5	10.7	9.9	3.7	tr	—
Acacia aroma (3)	8.0	1.5	18.5	3.7	13.5	3.5	7.6	0.3	4.7	0.2	3.3	1.2	3.2	6.7	10.9	9.5	3.6	tr	—
Acacia aroma (4)	8.3	1.5	18.9	3.6	12.8	3.7	7.8	0.3	4.8	0.3	3.2	1.2	3.0	6.9	10.5	9.3	3.8	tr	—
Acacia aroma (5)	8.1	1.6	18.1	3.5	12.7	3.6	7.9	0.3	4.9	0.3	3.1	1.2	3.2	6.8	10.8	9.9	3.9	tr	—
Acacia furcatispina (1)	11.9	1.6	26.5	1.4	22.4	1.5	13.0	1.1	7.8	0.3	5.1	0.3	3.0	1.0	1.1	1.0	0.9	tr	—
Acacia furcatispina (2)	11.8	1.6	27.0	1.5	22.0	1.5	13.0	1.1	7.7	0.3	5.2	0.3	3.1	1.0	1.0	1.0	0.8	tr	—
Acacia atramentaria (1)	6.9	1.2	15.5	1.6	10.9	1.9	7.5	2.3	4.7	1.9	4.6	4.3	3.5	6.4	12.6	9.2	4.9	tr	—
Acacia atramentaria (2)	7.2	1.3	15.8	1.6	10.7	1.8	7.3	2.3	4.5	1.8	4.7	4.4	3.6	6.7	12.3	9.1	4.8	tr	—
Acacia parviceps	7.6	0.1	17.4	0.2	15.7	0.2	9.9	0.5	6.6	0.3	3.5	9.2	6.2	9.4	4.2	8.9	tr	tr	—
Acacia horrida	16.7	0.6	27.6	0.5	19.5	0.5	11.4	0.5	7.3	tr	4.4	0.1	2.7	2.7	2.5	1.9	0.9	tr	—
Inga uruguensis	21.7	1.8	28.9	1.3	19.3	1.0	10.7	1.2	6.1	1.1	3.0	tr	1.5	tr	0.7	1.0	0.4	tr	—
Enterolobium contortisiliquum	11.2	1.1	25.2	1.8	18.8	2.0	11.8	2.1	7.4	0.2	5.7	0.4	3.9	1.1	7.1	tr	tr	tr	—
Tipuana tipu	18.9	0.8	32.4	0.7	20.2	0.4	10.9	0.5	6.2	tr	3.3	tr	2.6	0.4	0.9	0.6	0.9	tr	—
Sesbania punicea	10.7	2.3	16.2	2.0	9.0	2.5	5.9	2.5	4.2	4.0	3.8	6.7	5.2	10.3	6.9	6.8	0.9	tr	—
Indigofera parodiana	6.9	2.1	25.2	1.2	22.7	0.9	16.8	0.9	10.2	tr	5.5	tr	3.1	tr	2.6	tr	1.6	tr	—
Indigofera suffruticosa	19.8	0.4	33.2	0.5	21.0	tr	11.3	0.2	5.7	tr	2.7	tr	1.4	1.5	0.9	1.2	tr	tr	—
Desmodium cuneatum	12.1	0.4	27.7	0.4	22.9	0.3	12.9	0.3	7.4	tr	4.6	tr	3.1	0.5	2.1	2.1	1.2	1.9	—
Desmodium tortuosum	8.9	0.6	28.5	0.5	23.3	0.3	13.8	0.3	7.9	0.1	4.5	0.1	2.6	2.1	1.9	1.9	1.8	0.9	—
Desmodium neomexicanum	12.1	0.5	28.0	0.5	23.1	0.3	11.9	0.3	7.6	0.1	4.6	0.1	2.7	1.3	2.0	2.0	1.6	1.3	—
Collaea speciosa	14.7	2.2	31.0	1.3	19.9	1.2	11.7	0.4	6.7	tr	3.4	tr	1.9	tr	1.2	0.5	1.1	2.6	—
Galactia latisilicua (1)	8.9	0.2	27.7	0.3	24.6	0.1	14.8	0.2	8.0	tr	5.2	tr	4.3	0.5	1.6	3.0	0.5	tr	—
Galactia latisilicua (2)	8.5	0.1	27.9	0.3	24.2	0.1	14.6	0.1	8.3	tr	5.7	tr	3.8	0.8	1.9	2.9	0.5	tr	—
Galactia latisilicua (3)	8.6	0.1	26.8	0.3	24.2	0.1	14.0	0.2	8.1	tr	4.8	tr	5.0	0.3	3.2	3.0	0.5	tr	—
Cologania ovalifolia	14.4	0.8	15.6	1.0	33.1	1.1	12.2	0.5	7.3	tr	4.3	tr	2.9	2.9	1.8	1.8	tr	tr	—
Centrosema virginianum (1)	7.0	0.3	27.6	0.3	23.9	0.2	15.2	0.1	9.9	tr	6.2	tr	3.6	0.6	2.0	0.6	1.3	1.1	—
Centrosema virginianum (2)	6.7	2.4	26.9	2.8	21.2	1.9	12.8	0.6	8.8	tr	6.1	tr	3.9	1.1	2.4	0.8	1.3	tr	—
Rhynchosia edulis (1)	11.5	0.7	23.4	0.9	15.9	0.3	10.3	0.3	7.4	tr	5.3	tr	3.5	4.2	7.2	1.7	tr	tr	7.0
Rhynchosia edulis (2)	13.4	1.0	24.6	0.9	17.9	0.5	10.6	0.3	6.4	tr	4.4	tr	3.2	4.5	3.3	1.5	tr	tr	7.2
Rhynchosia edulis (3)	11.3	0.4	26.8	0.3	20.1	0.3	12.4	0.2	7.5	tr	4.0	tr	2.2	2.6	2.6	2.1	1.7	tr	5.2

Table 2—*continued.*

Rhynchosia edulis (4)	10.5	1.8	26.9	1.8	20.0	1.5	6.3	1.1	9.8	tr	5.6	tr	3.3	1.6	1.8	1.6	1.7	tr	4.5
Rhynchosia senna	10.4	0.2	26.5	0.2	20.3	0.1	11.8	0.1	6.3	tr	3.2	tr	1.6	5.5	2.9	2.5	1.2	tr	6.9
Aeschynomene rudis	20.3	1.6	31.7	1.0	19.1	0.8	10.4	0.9	5.9	tr	3.5	tr	2.5	tr	1.3	tr	0.8	tr	—
Stylosanthes montevidensis	6.8	0.7	26.3	1.5	25.2	1.0	16.3	0.8	9.7	tr	5.2	tr	3.0	tr	2.0	1.2	tr	tr	—
Stylosanthes gracilis	10.7	1.0	26.7	1.5	21.5	0.7	13.4	0.5	8.3	tr	4.8	tr	2.7	1.5	2.9	3.3	0.4	tr	—
Adesmia volckmanni	9.6	0.9	29.2	1.5	23.8	0.9	14.1	0.9	8.0	tr	4.3	tr	2.3	1.0	1.8	0.8	0.6	tr	—
Glycyrrhiza astragalina	14.5	1.0	28.3	1.0	19.2	0.9	10.7	0.7	8.4	tr	6.1	tr	3.9	0.2	2.3	1.6	0.9	tr	—
Lathyrus magellanicus	10.6	2.6	28.9	2.6	23.5	1.8	13.1	0.2	7.4	tr	4.2	tr	2.2	1.2	1.2	0.2	tr	tr	—

tr, trace less than 0.1%.

(CORD), and *Prosopis* specimens are deposited at the National Genebank of Prosopis (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba) (BNG).

Extraction of oil

Clean and dried samples of seeds were ground in a disintegrator. The powdered seeds were extracted with *n*-hexane in a Soxhlet apparatus for 12 h. The extracted oils were filtered and the solvent removed under reduced pressure in a rotary evaporator at 40°.

Isolation of unsaponifiable matter

The unsaponifiable fraction was obtained by direct saponification of the total lipids using methanolic KOH (IM) and recovered with $CHCl_3$.

Alkane isolation

The $CHCl_3$ soln of the unsaponifiable fraction was applied uniformly along a line 1.5 cm from one edge of (20 × 20 cm) 2 mm thickness plates coated with silica gel 60 G and developed three times with hexane–Et_2O (9 : 1) as developing solvent. The hydrocarbon zone was scraped from the plates and extracted with *n*-hexane. Further purification of the alkane fraction was carried out using $AgNO_3$–silica gel (1 : 9) prep. TLC using *n*-hexane–Et_2O (19 : 1) as developing solvent. IR ν_{max}^{KBr} cm^{-1}: 2825, 2854, 1481 and 1375.

GC, GC-MS

GC was performed using a CBPl (25 m × 0.25 mm i.d.) column programmed from 100°–300° at 5° min^{-1} using a FID with N_2 as carrier gas.

Mass spectrometric analysis was performed using a PERKIN ELMER Q MASS 910 gas chromatograph-mass spectrometer. A SE 30 (30 m × 0.25 mm i.d.) column was used with He (0.8 ml min^{-1}) as carrier gas and was temperature programmed from 60°–280° at 4° min^{-1}. Injector temperature at 290°. All spectra were obtained at 70 eV and the ion source temperature was 200°, with a filament current of 1 mA. Each peak was scanned over the range of 10–500 mass units.

The alkanes were identified by their IR spectra, comparisons of their gas chromatographic R_ts with respect to reference samples run under identical conditions and by their respective mass spectrometric fragmentation patterns.

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