

## HYDROCARBONS, STEROLS AND TOCOPHEROLS IN THE SEEDS OF SIX *ADANSONIA* SPECIES\*

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**Key Word Index**—*Adansonia*; Bombacaceae; baobab; hydrocarbons; sterols; tocopherols.

**Abstract**—The composition of the unsaponifiable matter of the lipids of six *Adansonia* species (*A. grandidieri*, *A. za*, *A. fony*, *A. madagascariensis*, *A. digitata* and *A. suarezensis*) was investigated. The total unsaponifiable content, its general composition and the identity of the components of the hydrocarbon, sterol and tocopherol fractions are presented. The unsaponifiable content in oil ranges from 0.4 to 1.1% (hexane method) and from 0.6 to 2.2% (diethyl ether method). In two species (*A. grandidieri* and *A. suarezensis*) the major components are 4-demethylsterols (23–42%) tocopherols (37–10%) and hydrocarbons (15–17%). In both species examined, eight 4-demethylsterols occur in the sterol fraction with sitosterol (81–88%) being predominant. Among the four tocopherols present,  $\gamma$ -tocopherol (68–98%) is the major compound. Each *Adansonia* species shows a characteristic gas liquid chromatography pattern for the hydrocarbon fraction. Squalene is the major component for five species (40–75%). Iso-, anteiso- and other branched hydrocarbons were not identified but were present in small amounts in comparison with *n*-alkanes. The dominance of odd- over even-carbon number chain length of *n*-alkanes was not observed in any species. The results show that  $C_{22}$ ,  $C_{25}$ ,  $C_{26}$ ,  $C_{27}$ ,  $C_{28}$  and  $C_{29}$  are the most frequent major constituents.

### INTRODUCTION

The genus *Adansonia* (baobab) of the Bombacaceae family including eight species and some intermediate forms, is well represented in the west part of Madagascar since six species are endemic [1]. The African species, *A. digitata* is also found in Madagascar and *A. gregorii* [2] is endemic to Australia.

It was reported in a previous paper that the 4-demethylsterol fraction of the African baobab seed oil was tentatively identified by GC [3]. Continuing with our work on the chemistry of the *Adansonia* genus [4] and our interest in ascertaining whether there exists any consistent relationship between the chemical composition and the taxonomical arrangement of this genus, has led us to a more extensive examination of the unsaponifiable lipid oils. In this study, nine seed oils coming from six *Adansonia* species (*A. grandidieri*, *A. za*, *A. fony*, *A. madagascariensis*, *A. digitata* and *A. suarezensis*) were investigated. CC and prep. TLC have been used to

fractionate the unsaponifiable matter into six fractions (hydrocarbons, tocopherols, 4,4-dimethylsterols, 4-methylsterols, 4-demethylsterols and unknown material). Among them, hydrocarbons, 4-demethylsterols and tocopherols were quantitatively analysed by GC.

### RESULTS

Baobab seeds are rather rich in oil (especially *A. grandidieri* and *A. suarezensis*) and they have been used for a long time in the production of vegetable oil by the people of the west coast of Madagascar. The oil content varies from one species to another and the results obtained by petrol extraction show that it ranges between 8 and 46% (Table 1). Among the methods described for the unsaponifiable lipid extraction, we have used those of Pelloquin *et al.* [5] with hexane and diethyl ether. The unsaponifiable lipid obtained using the diethyl ether method are slightly higher and more representative of the unsaponifiable matter (Table 1). In both cases, variable amounts were found for the samples of the different species. A good agreement was noticed between the values obtained for samples 1–3 of the same species of *A. grandidieri*.

\*Part 5 in the series "The Chemistry of the Malagasy Baobab". For Part 4 see ref [4]. Based on the Doctorate thesis presented by A. R. to the Université de Droit, d'Economie et des Sciences de Marseille (1980).

Table 1. Content of oils in dried seeds of six *Adansonia* species, percentage of unsaponifiable lipid in the oils and yields of six fractions obtained from the unsaponifiable lipid by column chromatography

Species	Content of oil in dried seeds (%)	Unsaponifiable in oil (%)		Fractions* from unsaponifiable (%)					
		Hexane	Et <sub>2</sub> O	1	2	3	4	5	6
<i>A. grandidieri</i> (sample 1)	38.7	0.52	0.89	—	—	—	—	—	—
<i>A. grandidieri</i> (sample 2)	36.4	0.54	0.93	15.5	37.1	13.2	9.4	23.7	1.0
<i>A. grandidieri</i> † (sample 3)	—	0.46	0.72	—	—	—	—	—	—
<i>A. za</i>	10.9	0.59	0.96	—	—	—	—	—	—
<i>A. fony</i>	10.5	0.92	1.34	—	—	—	—	—	—
<i>A. madagascariensis</i>	13.8	0.72	1.05	—	—	—	—	—	—
<i>A. digitata</i> (sample 1)	8.4	1.13	1.49	—	—	—	—	—	—
<i>A. digitata</i> ‡ (sample 2)	13.2	0.50	0.80	—	—	—	—	—	—
<i>A. suarezensis</i>	46.2	0.39	0.55	16.7	10.2	15.2	10.0	42.1	5.8

\*Fraction 1, hydrocarbons; fraction 2, tocopherols; fraction 3, 4, 4-dimethylsterols; fraction 4, 4-methylsterols; fraction 5, 4-demethylsterols; fraction 6, unknown.

†Industrial oil.

‡West Africa (upper Volta).

TLC examination of the unsaponifiable matter of the baobab seed oils showed many spots. The compound types were tentatively identified by comparison of the  $R_f$  values with standards (cholesterol, lanosterol,  $\alpha$ -tocopherol, squalene) these being in increasing order of  $R_f$  values: 4-demethylsterols, 4-methylsterols, 4,4-dimethylsterols, tocopherols and hydrocarbons. The relative content of each group of compounds from the unsaponifiable lipid was determined for *A. grandidieri* (sample 2) and *A. suarezensis* by CC on alumina [6]. The results obtained are given in Table 1. The separation of the different unsaponifiable fractions was also performed using prep. TLC on Si gel [7]. The bands of the different compounds of each group were located and scraped off the chromatoplate. To avoid interference between each group of components, two successive fractionations were made using prep. TLC. The percentage compositions of the 4-demethylsterols, tocopherols and hydrocarbons were determined by GC.

The sterols were analysed by GC as their acetates [8–11] and TMSi ether derivatives [3, 7, 12]. By comparison of  $R_f$ 's with standard sterols, we have tentatively identified seven of the eight sterols separated by GC. In all the samples, 24-ethylcholesterol, probably sitosterol (24  $\alpha$ -ethyl) was the major component with a content ranging from 77 to 88% (Table 2). The 24-methylcholesterol, presumably campesterol (24  $\alpha$ -methyl) and 24-ethylidene cholesterol (isofucosterol) (2–6%) were found at lower levels. Cholesterol, 24-ethylcholesta-5,22-dien-3 $\beta$ -ol (stigmasterol, 24  $\alpha$ -ethyl),  $\Delta^7$ -avenasterol and an unknown substance, with  $RR$ , 1.23, were encountered at a very much lower concentration in every sample.

Tocopherol identification was achieved by GC [13, 14]. The oil was saponified in the presence of pyrogallol and the unsaponifiable matter was extracted using the experimental conditions described by Pelloquin *et al.* [5]. The tocopherols were separated from the other compounds using prep. TLC as described

above. The chromatograms of the TMSi ethers of tocopherol derivatives involve three or four peaks. The products were tentatively identified by comparison with standard substances and with the literature data [14, 15]. The results are given in Table 3.

The analysis of the hydrocarbon fractions isolated by prep. TLC was made by GC according to the method described by Bastic *et al.* [16]. The products were tentatively identified both by comparison of their retention times with those of standards for the hydrocarbons ( $C_{12}$ – $C_{24}$ ) and by the method proposed by Guiochon [17] using the retention times from a programmed temperature analysis for the higher hydrocarbons ( $C_{25}$ – $C_{35}$ ). All the gas chromatograms showed several peaks representing saturated linear hydrocarbons or mono- or diunsaturated from  $C_{12}$  to  $C_{35}$  and branched *iso*- and *anteiso*-hydrocarbons. The results obtained for *n*-alkanes, *iso*- and *anteiso*-hydrocarbons and squalene are given in Table 4. The alkane fraction was obtained either from the hydrocarbon fraction by prep. TLC on silver nitrate–Si gel plates or from the whole seed oil using the method described by Faboya *et al.* [18]. Comparative results obtained by GC using two columns are given in Table 5 for one species of *Adansonia* (*A. za*). The alkane contents for the seed oils of six *Adansonia* species are given in Table 6.

## DISCUSSION

The results obtained for the unsaponifiable matter contained in the *Adansonia* seed oils show that there are significant differences between the compounds of each type for *A. grandidieri* (sample 2) and *A. suarezensis*. The 4-demethylsterol fraction represents more than 40% of the unsaponifiable lipid of *A. suarezensis* while it represents only 24% in *A. grandidieri*. For the latter sample, the major components are tocopherols (37%) (Table 1). The 4-demethylsterol fraction of all the oils investigated consists mainly of

Table 2. Composition of the 4-demethylsterol fractions of *Adansonia* seed oils

4-Demethylsterol	Oil samples*									
	<i>A. grandidieri</i>			<i>A. za A. fony A. madagascariensis</i>			<i>A. digitata</i>		<i>A. suarezensis</i>	
	<i>RR</i> , †	1	2	3	1	2	1	2	1	2
Cholesterol	0.61	0.6	0.8	tr	tr	tr	tr	0.1	1.9	0.4
24-Methylcholesterol (campesterol)	0.80	7.4	7.5	8.1	6.6	9.3	5.9	10.6	6.3	8.0
24-Ethyl-5, 22-cholesta- dien-3 $\beta$ -ol (stigma- sterol)	0.87	0.7	0.7	2.1	1.7	2.0	2.5	4.1	2.0	2.2
21-Ethylcholesterol (sitossterol)	1.00	86.5	87.0	85.1	88.4	83.5	85.8	76.6	81.0	85.0
24-Ethylidenecholesterol (isofucoesterol)	1.09	3.7	4.0	3.1	1.8	3.2	4.7	6.2	3.4	3.0
24-Ethyl-7-cholesten-3-ol ( $\Delta^7$ -stigmastenol)	1.16	0.3	tr	0.4	tr	0.8	1.1	0.5	4.5	0.4
Unknown	1.23	0.2	tr	0.3	0.1	0.3	tr	0.3	0.3	0.2
24-Ethylidene-7-cholesten- 3 $\beta$ -ol ( $\Delta^7$ -avenasterol)	1.28	0.6	tr	0.9	1.4	0.9	tr	1.6	0.6	0.8

tr, Denotes that component was detected in an amount too small to quantitate.

\*Area % by GC.

†Relative retention times of the TMSi ether derivatives of sterols on OV-17 SCOT glass capillary column (TMSi-sitosterol: 1.00).

Table 3. Composition of the tocopherol fractions of *Adansonia* seed oils

Tocopherol	RR <sub>i</sub> †	Oil samples*							
		<i>A. grandidieri</i>			<i>A. za</i>	<i>A. fony</i>	<i>A. madagascariensis</i>	<i>A. digitata</i>	<i>A. suarezensis</i>
		1	2	3					
δ—	0.53	2.5	6.1	0.5	tr	14.5	2.2	18.1	7.3
β—	0.63	7.6	25.6	0.5	2.4	29.1	3.8	12.0	3.0
γ—	0.67	89.9	68.3	98.7	97.6	56.4	92.2	59.7	89.0
α—	1.00	tr	tr	0.3	—	—	1.8	10.2	0.7

tr, Denotes that component was detected in an amount too small to quantitate.

\*Area % by GC.

†Relative retention times of the TMSi ether derivatives of tocopherols on OV-17 SCOT glass capillary column (TMSi-α-tocopherol: 1.00).

Table 4. Composition of the hydrocarbon fractions of *Adansonia* seed oils determined by GC\* analysis of the extracted unsaponifiable lipid

Hydrocarbon	Oil samples†							
	<i>A. grandidieri</i>			<i>A. za</i>	<i>A. fony</i>	<i>A. madagascariensis</i>	<i>A. digitata</i>	<i>A. suarezensis</i>
	1	2	3					
<i>n</i> -Alkanes	17.4	34.2	21.5	33.8	35.3	35.4	57.3	51.2
Iso-alkanes	1.6	1.0	0.7	—	1.7	4.4	1.5	1.8
Anteiso-alkanes	5.6	0.7	6.8	1.1	3.3	2.5	1.7	33.2
Squalene	75.4	64.1	70.9	65.0	59.7	57.7	39.5	13.7

—, Denotes that components were not detected.

\*OV-17 SCOT glass capillary column.

†Area % by GC.

Δ<sup>5</sup>-sterols while Δ<sup>7</sup>-sterols are present in smaller proportions (Table 2). These results are in good agreement with those previously described for the African *A. digitata* oil [3]. Cholesterol is present in low concentrations in all species. It is known that cholesterol occurs in the sterol fraction of many vegetable oils as a minor sterol component, although the sterol fraction of Cruciferae species contains a high proportion [19, 20] and a considerable amount of cholesten-7-enol as well as cholesterol was found in the 4-demethylsterol fraction of *Cordyline indivisa* seeds [21]. The sterol pattern in species of the same family is sometimes very different [9] and can be used for chemotaxonomical investigations. In the case of the *Adansonia* genus, the 4-demethylsterol composition is fairly constant from one species to another. 24-Ethylcholesterol is the main product in all cases, and it seems difficult to differentiate the species considering only the 4-demethylsterol fraction.

The tocopherol composition of baobab seed oil presents significant variations from one species to another as shown in Table 3. γ-Tocopherol is the main product in each case (56–99%). α-Tocopherol was not detected in *A. za* and *A. fony* and was found at a lower concentration in the other species al-

though in the case of *A. digitata* (sample 1) the content was 10%. β-Tocopherol was found at higher concentrations only in *A. fony*, (29%) and δ-tocopherol was found in variable amounts with the highest concentrations being in *A. fony* (14.5%) and *A. madagascariensis* (18%). Considerable interest has developed in the determination of tocopherols and the corresponding tocotrienols which display the activity of vitamin E [14, 22–24]. According to Green *et al.* [22] vegetable oils may be classified on the basis of their tocopherol content: those which contain α-, β- and γ-tocopherols and those which contain α-, γ- and δ-tocopherols. If we consider the results obtained for the three samples of *A. grandidieri*, the variation of β-, γ- and δ-tocopherols is rather large and the use of the tocopherol content to differentiate the species seems impracticable.

Long-chain hydrocarbons occurring in higher plants have been studied extensively and in many cases the composition of hydrocarbon fractions are characteristic and can be used as a taxonomic aid [25]. The universal presence of normal alkanes as constituents of leaf cuticular waxes has been discussed [26, 27]. However, hydrocarbons represent the least investigated fraction of the unsaponifiable mat-

Table 5. Comparison of the *n*-alkane compositions of the material recovered using two extraction methods on *A. za* seed oil

<i>n</i> -Alkane	From whole unsaponifiable		From whole seed oil	
	OV-17*	SE-30	OV-17	SE-30
C <sub>12</sub>	tr†	0.5	tr	tr
C <sub>13</sub>	tr	0.7	tr	tr
C <sub>14</sub>	0.5	0.6	tr	tr
C <sub>15</sub>	0.4	0.3	tr	tr
C <sub>16</sub>	1.4	1.0	tr	tr
C <sub>17</sub>	1.0	1.0	tr	tr
C <sub>18</sub>	3.1	2.6	1.4	1.6
C <sub>19</sub>	1.2	1.3	0.6	0.7
C <sub>20</sub>	1.5	3.2	3.0	2.6
C <sub>21</sub>	1.5	2.2	3.3	1.4
C <sub>22</sub>	17.3	13.7	19.0	19.8
C <sub>23</sub>	4.4	4.4	5.6	4.2
C <sub>24</sub>	5.9	4.9	7.4	7.4
C <sub>25</sub>	8.6	9.1	10.3	10.2
C <sub>26</sub>	9.4	8.2	11.4	10.5
C <sub>27</sub>	13.4	9.7	12.0	13.5
C <sub>28</sub>	7.7	9.6	9.3	8.9
C <sub>29</sub>	11.6	10.9	7.3	9.1
C <sub>30</sub>	4.2	5.3	3.4	4.3
C <sub>31</sub>	4.3	8.0	2.8	3.9
C <sub>32</sub>	1.7	1.8	1.4	1.8
C <sub>33</sub>	1.0	1.0	0.7	tr
C <sub>34</sub>	tr	tr	0.5	tr
C <sub>35</sub>	tr	tr	0.6	tr

tr, Denotes component was detected in an amount too small to quantify.

\*For conditions employed for the GC analysis see the Experimental.

†Area % by GC.

ter of vegetable oils [16]. Hydrocarbon fractions of the six *Adansonia* species seed oils were found to contain *n*-alkanes, squalene and various branched hydrocarbons. *Iso*- and *anteiso*-alkanes were tentatively identified but represent a small proportion of the hydrocarbon fraction as shown in Table 4. The squalene composition of the oils showed differences which could perhaps be used for the characterization of *A. suarezensis*, where this compound comprises only 14% of the hydrocarbon fraction. We found that the two methods to obtain the *n*-alkane fraction by GC using two columns gave similar results (Table 5). Herbin and Robins [28] have shown that the predominating *n*-alkanes of the leaf cuticular waxes are odd-carbon number constituents such as nonacosane (C<sub>29</sub>) and hentriacontane (C<sub>31</sub>) and even-carbon constituents are less significant. Odd-carbon number compounds predominate in the leaf waxes of *Khaya* species [18]. The results obtained for the six *Adansonia* species show a significant proportion of *n*-alkanes between C<sub>20</sub> and C<sub>31</sub> with a predominance of C<sub>22</sub> (5–19%), C<sub>25</sub> and C<sub>26</sub> (4–16%), C<sub>27</sub> (10–14%) and C<sub>28</sub> and C<sub>29</sub> (4–15%) as shown in Table 6. The preponderance of odd-carbon number compounds was not observed. Similar results were described by Bastic *et al.* [16] for some oils, especially soya bean oil.

Kaneda [29] in a study of the hydrocarbons occurring in the external and internal lipids of fresh spinach leaves found that the external lipids contain normal paraffin: *n*-C<sub>27</sub>–*n*-C<sub>33</sub> showing a clear odd-number preference but the internal lipids contain a significant proportion of *n*-C<sub>16</sub>–*n*-C<sub>28</sub> paraffin with no bias to odd-number compounds.

## EXPERIMENTAL

**Unsaponifiable lipid extraction.** The origin and collection of the baobab fruits was described in ref. [4]. The seed oils were prepared from the corresponding dried crushed seeds by Soxhlet extraction with petrol (40–60°). *A. grandidieri* (sample 3) seed oil was factory-prepared oil. Authentic specimens of  $\alpha$ -tocopherol, squalene, lanosterol, cholesterol, stigmasterol and sitosterol and a sterol fraction consisting of campesterol, stigmasterol, sitosterol, isofucoesterol,  $\Delta^7$ -stigmasterol and  $\Delta^7$ -avenasterol were used as standards for prep. TLC and GC. Saponification and extraction of the unsaponifiable lipids using hexane or Et<sub>2</sub>O was performed as described previously [5].

**Column chromatography.** The unsaponifiable extract (500 mg) was fractionated on a column of alumina, Brockmann grade II–III (120 g) [6] using the following solvent mixtures: 200 ml hexane; 200 ml hexane–C<sub>6</sub>H<sub>6</sub> (5:5); 200 ml

Table 6. Composition of the *n*-alkanes determined by GC\* analysis of the whole seed oils of *Adansonia* species

<i>n</i> -Alkane†	Oil samples						
	<i>A. grandidieri</i>		<i>A. za</i>	<i>A. fony</i>	<i>A. madagascariensis</i>	<i>A. digitata</i>	<i>A. suarezensis</i>
	1	3					
C <sub>12</sub>	tr	tr	tr	tr	tr	tr	tr
C <sub>13</sub>	tr	tr	tr	tr	tr	0.5	tr
C <sub>14</sub>	tr	1.6	tr	tr	0.1	1.2	1.0
C <sub>15</sub>	tr	0.4	tr	tr	0.1	0.2	tr
C <sub>16</sub>	0.8	0.7	tr	tr	2.3	13.1	tr
C <sub>17</sub>	0.7	0.6	tr	tr	1.2	0.5	tr
C <sub>18</sub>	2.1	0.6	1.4	tr	5.1	22.7	4.3
C <sub>19</sub>	0.7	0.9	0.6	tr	1.0	0.5	0.6
C <sub>20</sub>	2.4	1.7	3.0	0.6	2.2	1.4	7.1
C <sub>21</sub>	2.6	2.9	3.3	2.1	7.3	0.6	3.2
C <sub>22</sub>	13.6	6.9	19.0	15.4	9.7	5.0	10.4
C <sub>23</sub>	7.2	9.7	5.6	6.4	11.6	1.5	5.7
C <sub>24</sub>	7.5	6.7	7.4	8.6	11.5	2.4	10.0
C <sub>25</sub>	10.6	9.1	10.3	11.9	9.4	4.0	11.8
C <sub>26</sub>	11.7	11.2	11.4	15.7	13.3	5.1	12.5
C <sub>27</sub>	10.1	13.2	12.0	14.2	10.4	13.4	11.2
C <sub>28</sub>	8.8	11.3	9.3	8.5	5.6	6.3	5.9
C <sub>29</sub>	8.3	14.9	7.3	9.4	5.4	11.2	4.2
C <sub>30</sub>	7.6	5.2	3.4	3.2	2.4	3.1	3.7
C <sub>31</sub>	3.0	1.2	2.8	2.2	0.6	4.4	5.2
C <sub>32</sub>	1.5	1.2	1.4	1.3	0.5	1.4	2.1
C <sub>33</sub>	0.8	tr	0.7	0.6	0.3	1.4	1.2
C <sub>34</sub>	tr	tr	0.5	tr	tr	tr	tr
C <sub>35</sub>	tr	tr	0.6	tr	tr	tr	tr

tr, Denotes component was detected in an amount too small to quantitate.

\*OV-17 SCOT glass capillary column.

†Area % by GC.

hexane-C<sub>6</sub>H<sub>6</sub> (2:8); 500 ml hexane-Et<sub>2</sub>O (5:5); 500 ml Et<sub>2</sub>O; and 250 ml MeOH. Fractions (20 ml) were collected and each checked using Si gel TLC.

**Preparative TLC of the unsaponifiable matter.** The approximate *R<sub>f</sub>* values of compounds on Si gel TLC developed with CHCl<sub>3</sub>-Et<sub>2</sub>O (9:1) were: hydrocarbons and squalene, 0.97;  $\alpha$ -tocopherol and other tocopherols, 0.66; lanosterol and other 4, 4-dimethylsterols, 0.45; 4-methylsterols, 0.35; cholesterol and other 4-demethylsterols, 0.27. The unsaponifiable lipid was dissolved in CCl<sub>4</sub> and 150  $\mu$ l applied as a streak of 15 cm length to a Si gel chromatoplate. Squalene,  $\alpha$ -tocopherol, lanosterol and cholesterol were also spotted as markers. After development the standards were visualized with Rhodamine B under UV at 366 nm and the corresponding bands of hydrocarbons, tocopherols and 4-demethylsterols were scraped off the chromatoplate and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The whole hydrocarbon fraction was separated into saturated alkanes (paraffins) and olefins by prep. TLC on AgNO<sub>3</sub>-Si gel plates. After development with C<sub>6</sub>H<sub>6</sub>, the alkane band was scraped off the plate, extracted with hexane and analysed by GC.

**Isolation of the alkane fractions from the whole seed oil.** Seed oil (500 mg) was dissolved in cyclohexane (5 ml), separated on a dry column of activated alumina (2 hr at 105°) and eluted with cyclohexane. The first 5 ml of eluate was collected and evaporated to dryness. Prep. TLC on AgNO<sub>3</sub>-Si gel plates was used to separate alkanes from olefinic compounds as described above.

**GC analysis.** 4-Demethylsterols and tocopherols were analysed as their TMSi ether derivatives using an OV-17 SCOT glass capillary column (30 m  $\times$  0.36 mm, 250°, 0.25  $\mu$ m phase thickness, sample vol. 1-2  $\mu$ l). The *RR<sub>i</sub>* for the TMSi-derivatives of 4-demethylsterols and tocopherols were calculated relative to the sitosterol and  $\alpha$ -tocopherol derivatives respectively. Hydrocarbons were chromatographed on a steel column (1.5 m  $\times$  3 mm) packed with 5% SE-30 on Gas Chrom Q. The temp. was programmed from 150° to 310° at 3°/min; N<sub>2</sub> at 40 ml/min. Hydrocarbons were also chromatographed on an OV-17 SCOT glass capillary column (30 m  $\times$  0.36 mm, 0.25  $\mu$ m phase thickness). The temp. was programmed from 150° to 290° at 5°/min.

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