

# Hydrocarbons from Three *Vanilla* Bean Species: *V. fragrans*, *V. madagascariensis*, and *V. tahitensis*

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The hydrocarbon content, isolated by column chromatography over alumina gel, in three *Vanilla* bean species (*V. fragrans*, *V. madagascariensis*, and *V. tahitensis*) ranges between 0.2 and 0.6%. The composition of these hydrocarbon fractions has been investigated. Using retention indices and gas chromatography/mass spectrometry, 25 *n*-alkanes, 17 branched alkanes, and 12 alkenes have been identified. The major constituents are odd-numbered hydrocarbons including *n*-nonacosane and *n*-hentriacontane. The major even-numbered compounds are *n*-octacosane and *n*-triacontane. Since *V. fragrans* from Reunion Island contains large amounts of *n*-alkanes and *n*-alkenes, and *V. tahitensis* is rich in branched alkanes, hydrocarbon profiles could be used for origin differentiation.

**Keywords:** *Vanilla* beans; *V. fragrans*; *V. madagascariensis*; *V. tahitensis*; hydrocarbons; *n*-alkanes; *n*-1-alkenes; GC/MS

## INTRODUCTION

Vanilla flavor is well-known and appreciated in the world and is widely used in the food industry. This flavor comes from two principal *Vanilla* species: *V. fragrans* and *V. tahitensis*. Although the cultivated vanilla from Madagascar belongs to the *V. fragrans* species (Ranadive, 1992), there is in this island a native species, *V. madagascariensis*, which does not give a characteristic vanilla flavor after the drying and curing process. Since the last century, the identification of vanilla volatile compounds elicited a lot of studies and >200 compounds were identified (Klimes and Lamparsky, 1976; Hartman *et al.*, 1992; Ehlers *et al.*, 1994, 1995). However, the lipidic fraction of vanilla beans, which also contributes to the vanilla aroma, has not been extensively studied.

The Orchidaceae family has been studied by Holman and Nichols (1972), especially for their hydrocarbon composition of the lipidic fraction from plants belonging to the genus *Phalaenopsis*. The hydrocarbon fraction generally contains alkanes, alkenes, sesquiterpenes, diterpenes, and triterpenes. Eglinton and Hamilton (1963) have used the nature and composition of the hydrocarbon fraction as the basis for a taxonomical study. This fraction has been studied in many vegetable oils (Bastic *et al.*, 1978). Because the composition of the hydrocarbon fraction from *Vanilla* has not been reported, we have decided to study three *Vanilla* species: *V. fragrans* (collected in Reunion Island, known as Bourbon vanilla), *V. madagascariensis* (collected in Madagascar), and *V. tahitensis* (collected in Tahiti).

## MATERIALS AND METHODS

**Origin of Vanilla Beans.** *V. madagascariensis* (native species from Madagascar) was collected in the Antalaha area (northeast Madagascar). The two other species, *V. fragrans*

and *V. tahitensis*, were collected in Reunion and Tahiti Islands, respectively.

**Unsaponifiable Extraction.** Neutral lipids were obtained from crushed beans (40 g) using Soxhlet extraction with pentane (150 mL, 16 h). The unsaponifiable fraction was obtained using the procedure described by Itoh *et al.* (1973). Water (40 mL) was added to the reaction mixture (10 mL), and unsaponifiable matter was extracted with diisopropyl ether (3 × 30 mL). The organic layer was washed with a 10% aqueous carbonate solution (3 × 4 mL) and then with distilled water to neutrality, dried, and evaporated (19.5, 20.8, and 31.9% yield for *V. tahitensis*, *V. madagascariensis*, and *V. fragrans*, respectively).

**Column Chromatography.** The unsaponifiable extract (0.425 and 0.525 g from *V. fragrans* and *V. madagascariensis* beans, respectively) was fractionated by column chromatography (CC; 46 × 3 cm) over alumina gel (Aluminoxid 90 II-III, Merck 1097, 70-230 mesh ASTM, 3–6% hydration) using hexane (400 mL). Fractions (25 mL) were collected, and each was checked using thin-layer chromatography (TLC) on pre-coated plates (5 × 10 cm, silica gel 60 F<sub>254</sub>, 0.25 mm, Merck), eluent CHCl<sub>3</sub>/Et<sub>2</sub>O (90:10, v/v). Spots were visualized using phosphomolybdic acid spray reagent. Tubes 1–9 contained hydrocarbons (approximate *R<sub>f</sub>* values 0.84–0.90). For *V. fragrans*, the hydrocarbon fraction represented 15.6, 4.9, and 0.43% with respect to unsaponifiable materials, lipids, and beans, respectively; the values for *V. madagascariensis* were 7.0, 1.4, and 0.19%.

**Preparative TLC.** The relative content of hydrocarbons from the unsaponifiable lipid of *V. tahitensis* was determined by preparative TLC on pre-coated plates (10 × 20 cm, silica gel 60 F<sub>254</sub>, 0.25 mm, Merck). The unsaponifiable lipid (10 mg) was dissolved in CCl<sub>4</sub> and applied as a streak to the Si gel plate. Cholesterol was also spotted as a marker. After development with CHCl<sub>3</sub>/Et<sub>2</sub>O (90:10, v/v), the spots were visualized with Rhodamine B (250 mg in ethanol, 150 mL) under ultraviolet light at 254 nm. The corresponding band of hydrocarbons was scraped off the plate and extracted with CH<sub>2</sub>Cl<sub>2</sub> (10 × 1 mL). Mass percentages of hydrocarbons of *V. tahitensis* were 47.5, 9.2, and 0.6% from unsaponifiable materials, lipids, and beans, respectively.

**Gas Chromatography (GC).** A Girdel 30 gas chromatograph equipped with a flame ionization detector (FID) was used for compound separations with an OV-1 glass capillary column (25 m × 0.31 mm i.d.) (phase thickness, 0.15 μm; column temperature, 70–220 °C, 3 °C min<sup>-1</sup>). Detector and inlet temperatures were 300 and 295 °C, respectively. Hydrogen was used as a carrier gas at an inner pressure of 0.5

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**Table 1. Percentage of Neutral Lipid, Unsaponifiable Matter, and Hydrocarbon Content in the Three *Vanilla* Beans Species under Study**

<i>Vanilla</i> species	neutral lipid content <sup>a</sup> (%) in beans	unsaponifiable matter (%) in neutral lipid fraction <sup>b</sup>	hydrocarbon content (%) in		
			unsaponifiable matter <sup>c</sup>	neutral lipid	beans
<i>V. fragrans</i>	10.8 <sup>e</sup>	31.9 <sup>e</sup>	15.6	4.9	0.4
<i>V. madagascariensis</i>	14.0	20.8	7.0	1.4	0.2
<i>V. tahitensis</i>	9.3	19.5	47.5 <sup>d</sup>	9.2	0.6

<sup>a</sup> Percentage based on dry material ( $\pm 0.5\%$ ). <sup>b</sup> Mass percentage expressed with respect to neutral lipids ( $\pm 0.5\%$ ). <sup>c</sup> Obtained by CC over alumina gel III-II for *V. fragrans* and *V. madagascariensis*. <sup>d</sup> Obtained by preparative TLC for *V. tahitensis*. <sup>e</sup> Average for three samples.

**Table 2. *n*-Alkane Distribution in the Hydrocarbon Fraction of the Three *Vanilla* Bean Species under Study**

no.	formula	compound	$I_R^{a,b}$	relative composition <sup>a</sup> (%)		
				<i>V. fragrans</i>	<i>V. tahitensis</i>	<i>V. madagascariensis</i>
1	C <sub>10</sub> H <sub>22</sub>	<i>n</i> -decane	1000		0.6	
2	C <sub>12</sub> H <sub>26</sub>	<i>n</i> -dodecane	1200		0.6	
3	C <sub>14</sub> H <sub>30</sub>	<i>n</i> -tetradecane	1400	0.1	0.4	1.0
4	C <sub>15</sub> H <sub>32</sub>	<i>n</i> -pentadecane	1500	0.1	0.2	0.2
5	C <sub>16</sub> H <sub>34</sub>	<i>n</i> -hexadecane	1600	0.8	2.4	6.0
6	C <sub>17</sub> H <sub>36</sub>	<i>n</i> -heptadecane	1700	0.4	0.4	0.3
7	C <sub>18</sub> H <sub>38</sub>	<i>n</i> -octadecane	1800	1.0	2.9	4.1
8	C <sub>19</sub> H <sub>40</sub>	<i>n</i> -nonadecane	1900	0.8	7.9	0.2
9	C <sub>20</sub> H <sub>42</sub>	<i>n</i> -eicosane	2000	0.6	2.2	1.8
10	C <sub>21</sub> H <sub>44</sub>	<i>n</i> -heneicosane	2100	1.4	1.8	1.7
11	C <sub>22</sub> H <sub>46</sub>	<i>n</i> -docosane	2200	2.9	4.6	3.4
12	C <sub>23</sub> H <sub>48</sub>	<i>n</i> -tricosane	2300	15.3	7.8	15.2
13	C <sub>24</sub> H <sub>50</sub>	<i>n</i> -tetracosane	2400	8.6	4.0	8.4
14	C <sub>25</sub> H <sub>52</sub>	<i>n</i> -pentacosane	2500	21.9	9.0	14.9
15	C <sub>26</sub> H <sub>54</sub>	<i>n</i> -hexacosane	2600	5.5	2.3	7.1
16	C <sub>27</sub> H <sub>56</sub>	<i>n</i> -heptacosane	2700	10.8	7.5	9.0
17	C <sub>28</sub> H <sub>58</sub>	<i>n</i> -octacosane	2800	4.5	2.7	3.5
18	C <sub>29</sub> H <sub>60</sub>	<i>n</i> -nonacosane	2900	10.3	12.8	6.9
19	C <sub>30</sub> H <sub>62</sub>	<i>n</i> -triacontane	3000	3.5	10.8	2.8
20	C <sub>31</sub> H <sub>64</sub>	<i>n</i> -hentriacontane	3100	8.1	6.0	3.9
21	C <sub>32</sub> H <sub>66</sub>	<i>n</i> -dotriacontane	3200	1.0	1.7	1.0
22	C <sub>33</sub> H <sub>68</sub>	<i>n</i> -tritriacontane	3300	1.0	0.7	1.0
23	C <sub>34</sub> H <sub>70</sub>	<i>n</i> -tetraatriacontane	3400	0.7	3.1	0.3
24	C <sub>35</sub> H <sub>72</sub>	<i>n</i> -pentatriacontane	3500	0.5	1.9	0.2
25	C <sub>36</sub> H <sub>74</sub>	<i>n</i> -hexatriacontane	3600	0.2	4.9	0.1
		$\Sigma$ even <sup>c</sup>		29.4	43.2	46.7
		$\Sigma$ odd <sup>d</sup>		70.6	56.8	53.5

<sup>a</sup> Percentage based on dry material ( $\pm 0.5\%$ ). <sup>b</sup> Mass percentage expressed with respect to neutral lipids ( $\pm 0.5\%$ ). <sup>c</sup> Obtained by CC over alumina gel III-II for *V. fragrans* and *V. madagascariensis*. <sup>d</sup> Obtained by preparative TLC for *V. tahitensis*. <sup>e</sup> Average for three samples.

bar (3 mL/min, split 60 mL/min). The injections averaged 1  $\mu$ L of a 0.5% solution of crude mixtures in hexane.

**Gas Chromatography/Mass Spectrometry (GC/MS).** Combined GC/MS was carried out on a Girdel gas chromatograph linked to a Ribermag R-10-10B mass spectrometer equipped with a quadrupole mass analyzer (15.6 mm i.d.  $\times$  350 mm,  $10^{-6}$  mmHg) and coupled with a Sidar data computer. The GC column was an OV-1701 fused capillary column (50 m  $\times$  0.32 mm, 0.30  $\mu$ m phase thickness), and the following conditions were used: column temperature, 100–280 °C; 3 °C min<sup>-1</sup>; carrier gas, helium (2 bar, 4 mL/min, split 80 mL/min); ion source, 270 °C; ionizing voltage, 70 eV.

## RESULTS AND DISCUSSION

The lipid content of the three *Vanilla* bean species of various origins is presented in Table 1. It varies from one species to another, and the results obtained by pentane extraction show that it ranges from 9.3 to 14.0%. These values are higher than those published in the literature (Holman and Nichols, 1972) for other Orchidaceae. In fact, the lipid content has been reported to account for 0.7–1.3% in the *Phalaenopsis* genus, 0.5–0.7% in the *Cattleya* genus, and 0.6–1.1% in the *Cymbidium* genus. However, in the *Vanilla* genus, *V. madagascariensis* is distinguishable from the other two by its high lipid content (14%), whereas the lowest content is found in Tahitian beans (9.3%).

Among the methods described for the unsaponifiable extraction, we have used the procedure described by Itoh *et al.* (1973). The unsaponifiable lipids obtained range from 19.5 to 31.9% (Table 1) for the three species, without any significant difference for *V. tahitensis* (19.5%) and *V. madagascariensis* (20.8%). In any case, we note that the unsaponifiable lipid content of vanilla beans is higher than that of edible vegetable oils such as olive oil (0.8–1.3%; Hilditch and Thompson, 1937; Hilditch and Maddison, 1941) or peanut oil (0.5%; Pelloquin *et al.*, 1977). The hydrocarbon fraction was obtained from the different unsaponifiable fractions by liquid chromatography over alumina (CC) for *V. fragrans* and *V. madagascariensis* and by preparative thin-layer chromatography (TLC) for *V. tahitensis*. The results obtained are given in Table 1. *V. madagascariensis* is characterized by a lower hydrocarbon range (7.0, 1.4, and 0.2% from unsaponifiable portion, lipids, and beans, respectively). For *V. fragrans*, we have found 15.6, 4.9, and 0.4%, respectively, and 47.5, 9.2, and 0.6% for *V. tahitensis*. Although the unsaponifiable matter is relatively high in *V. fragrans*, compared to the other two species, the hydrocarbon content in beans, for the three species, is quite stable and ranges from 0.2 to 0.6%. Furthermore, the hydrocarbon content of the *Vanilla* genus is higher than that of edible oils given

**Table 3. 3-Methylalkane Distribution in the Hydrocarbon Fraction of the Three *Vanilla* Bean Species under Study**

no.	formula	compound	$I_R^{a,b}$	relative composition <sup>a</sup> (%)		
				<i>V. fragrans</i>	<i>V. tahitensis</i>	<i>V. madagascariensis</i>
<b>26</b>	C <sub>16</sub> H <sub>34</sub>	3-methylpentadecane	1565	0.4	0.3	0.2
<b>27</b>	C <sub>18</sub> H <sub>38</sub>	3-methylheptadecane	1765	0.8	0.4	0.1
<b>28</b>	C <sub>20</sub> H <sub>42</sub>	3-methylnonadecane	1965	1.6	0.5	0.1
<b>29</b>	C <sub>21</sub> H <sub>44</sub>	3-methyleicosane	2065	2.0	0.6	0.2
<b>30</b>	C <sub>23</sub> H <sub>48</sub>	3-methyltricosane	2265	34.7	11.4	64.3
<b>31</b>	C <sub>25</sub> H <sub>52</sub>	3-methyltetracosane	2465	40.9	26.4	26.4
<b>32</b>	C <sub>27</sub> H <sub>56</sub>	3-methylhexacosane	2665	14.5	54.2	6.4
<b>33</b>	C <sub>32</sub> H <sub>66</sub>	3-methylhentriacontane	3165	3.1	5.0	2.1
<b>34</b>	C <sub>35</sub> H <sub>72</sub>	3-methyltritriacontane	3465	2.0	1.2	0.2
		Σeven <sup>c</sup>		5.9	6.2	2.5
		Σodd <sup>d</sup>		94.1	93.8	97.5

<sup>a</sup> Determined on a 25 m capillary column (OV-1). <sup>b</sup> Retention indices. <sup>c</sup> Sum for 3-methylalkanes with even number of carbon atoms. <sup>d</sup> Sum for 3-methylalkanes with odd number of carbon atoms.

**Table 4. 5-Ethylalkane Distribution in the Hydrocarbon Fraction of the Three *Vanilla* Bean Species under Study**

no.	formula	compound	$I_R^{a,b}$	relative composition <sup>a</sup> (%)		
				<i>V. fragrans</i>	<i>V. tahitensis</i>	<i>V. madagascariensis</i>
<b>35</b>	C <sub>16</sub> H <sub>34</sub>	5-ethyltetradecane	1568	0.5	0.4	2.6
<b>36</b>	C <sub>18</sub> H <sub>38</sub>	5-ethylhexadecane	1768	1.0	0.8	2.8
<b>37</b>	C <sub>20</sub> H <sub>42</sub>	5-ethyloctadecane	1968	1.5	1.0	6.3
<b>38</b>	C <sub>27</sub> H <sub>56</sub>	5-ethylpentacosane	2668	9.5	10.0	50.4
<b>39</b>	C <sub>29</sub> H <sub>60</sub>	5-ethylheptacosane	2868	22.3	18.4	20.4
<b>40</b>	C <sub>31</sub> H <sub>64</sub>	5-ethylnonacosane	3068	26.2	41.5	10.1
<b>41</b>	C <sub>33</sub> H <sub>68</sub>	5-ethylhentriacontane	3268	36.4	25.9	5.2
<b>42</b>	C <sub>35</sub> H <sub>72</sub>	5-ethyltritriacontane	3468	2.5	2.0	2.2
		Σeven <sup>c</sup>		3.0	2.2	11.7
		Σodd <sup>d</sup>		97.0	97.8	88.3

<sup>a</sup> Determined on a 25 m capillary column (OV-1). <sup>b</sup> Retention indices. <sup>c</sup> Sum for 5-ethylalkanes with even number of carbon atoms. <sup>d</sup> Sum for 5-ethylalkanes with odd number of carbon atoms.

**Table 5. Alkene Distribution in the Hydrocarbon Fraction of the Three *Vanilla* Bean Species under Study**

no.	formula	compound	$I_R^{a,b}$	relative composition <sup>a</sup> (%)		
				<i>V. fragrans</i>	<i>V. tahitensis</i>	<i>V. madagascariensis</i>
<b>43</b>	C <sub>14</sub> H <sub>28</sub>	1-tetradecene	1388			0.2
<b>44</b>	C <sub>16</sub> H <sub>32</sub>	1-hexadecene	1588	0.8	0.2	1.6
<b>45</b>	C <sub>18</sub> H <sub>36</sub>	1-octadecene	1788	1.4	0.1	1.7
<b>46</b>	C <sub>20</sub> H <sub>40</sub>	1-eicosene	1988	0.6	0.9	0.9
<b>47</b>	C <sub>22</sub> H <sub>44</sub>	1-docosene	2188	0.6	0.8	0.5
<b>48</b>	C <sub>23</sub> H <sub>46</sub>	1-tricosene	2288	2.0	1.0	0.9
<b>49</b>	C <sub>25</sub> H <sub>50</sub>	1-pentacosene	2488	0.6	2.0	14.2
<b>50</b>	C <sub>27</sub> H <sub>54</sub>	1-heptacosene	2688	1.4	21.1	1.3
<b>51</b>	C <sub>29</sub> H <sub>58</sub>	1-nonacosene	2888	25.2	23.2	19.4
<b>52</b>	C <sub>31</sub> H <sub>62</sub>	1-hentriacontene	3088	55.3	38.5	50.5
<b>53</b>	C <sub>32</sub> H <sub>64</sub>	1-dotriacontene	3188	0.7	0.4	1.2
<b>54</b>	C <sub>33</sub> H <sub>66</sub>	1-tritriacontene	3288	11.4	11.8	7.6
		Σeven <sup>c</sup>		3.5	2.4	6.1
		Σodd <sup>d</sup>		96.5	97.6	93.9

<sup>a</sup> Determined on a 25 m capillary column (OV-1). <sup>b</sup> Retention indices. <sup>c</sup> Sum for *n*-1-alkenes with even number of carbon atoms. <sup>d</sup> Sum for *n*-1-alkenes with odd number of carbon atoms.

by some authors [between 0.1 and 1.0% in Bastic *et al.* (1978) and Capella *et al.* (1963)]. Hydrocarbon identification was achieved by GC/MS (McLafferty, 1969) and by comparison of their retention indices ( $I_R$ ) with the standards (Jennings, 1980), using the formula  $I_R = 100N + X$  where  $X = 0$  for *n*-alkanes,  $X = 65$  for 3-methylalkanes,  $X = 68$  for 5-ethylalkanes, and  $X = 88$  for *n*-1-alkenes. Hydrocarbons from the three *Vanilla* species were found to contain *n*-alkanes (**1–25**), C<sub>10–36</sub> (Table 2), 3-methylalkanes (**26–34**), C<sub>16–34</sub> (Table 3), 5-ethylalkanes (**35–42**), C<sub>16–35</sub> (Table 4), and *n*-1-alkenes (**43–54**), C<sub>14–33</sub> (Table 5). The presence of some saturated, monounsaturated, and branched hydrocarbons has already been reported in methanolic and ethanolic vanilla bean extracts by Klimes and Lamparsky (1976) but only for compounds up to 20 carbon atoms. Linear alkanes were identified on the basis of their mass spectra and by cochromatography with

authentic *n*-alkanes. The results revealed the presence of saturated hydrocarbons in the series C<sub>10–36</sub> for *V. fragrans* and *V. tahitensis* and in the series C<sub>14–36</sub> for *V. madagascariensis*. In the *n*-alkane series, odd-numbered constituents such as *n*-pentacosane (**14**) (21.9%) in *V. fragrans* or *n*-nonacosane (**18**) (12.8%) in *V. tahitensis* were predominant. These results are in agreement with those described by Herbin and Robins (1968). GC/MS analyses revealed 17 methyl-branched saturated hydrocarbons (compounds **26–34**) and ethyl-branched saturated hydrocarbons (compounds **35–42**). In the 3-methylalkane series, odd-numbered constituents were most important (88–98%). The major compounds were 3-methyltricosane (**30**) for *V. madagascariensis* (64.3%), 3-methyltetracosane (**31**) for *V. fragrans* (40.9%), and 3-methylhexacosane (**32**) for *V. tahitensis* (54.2%). 5-Ethylalkanes were well represented with 5-ethylpentacosane (**30**) in *V. madagascariensis* (50.4%),

**Table 6. Distribution of the Various Hydrocarbon Families in the Three *Vanilla* Beans Species under Study**

family	relative composition <sup>a</sup> (%)		
	<i>V. fragrans</i>	<i>V. tahitensis</i>	<i>V. madagascariensis</i>
<i>n</i> -alkanes	45.8	12.0	28.2
3-methylalkanes	15.2	46.8	36.5
5-ethylalkanes	12.8	33.0	17.8
<i>n</i> -1-alkenes	26.2	8.2	17.5

<sup>a</sup> Determined on a 25 m capillary column (OV-1).

5-ethylnonacosane (**40**) in *V. tahitensis* (41.5%), and 5-ethylhentriacontane (**41**) in *V. fragrans* (36.4%). *n*-1-Alkenes **43–54** in the series C<sub>14–33</sub> have also been identified. Odd-numbered constituents predominated, and for the three species investigated, 1-hentriacontene (**52**) was the main compound.

The study of the distribution of the various hydrocarbon families (Table 6) shows that *n*-alkanes and *n*-alkenes are the two main hydrocarbon families in *V. fragrans* (45.8 and 26.2%, respectively). In the case of *V. tahitensis*, branched alkanes represent >79% of the hydrocarbon fraction. Therefore, hydrocarbon profiles can be used for differentiation of vanilla flavorings from Reunion (Bourbon vanilla) and from Tahiti. The hydrocarbon profile of *V. madagascariensis* is not as characteristic as that of the two other species.

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