Long-Chain Aliphatic β-Diketones from Epicuticular Wax of Vanilla Bean Species. Synthesis of Nervonoylacetone

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · NOVEMBER 2000

Impact Factor: 2.91 · DOI: 10.1021/jf0002701 · Source: PubMed

3 AUTHORS:

Béatrice Ramaroson-Raonizafinimanana
University of Antananarivo, Ecole Supérieure…
6 PUBLICATIONS **42** CITATIONS
SEE PROFILE

Emile M Gaydou
Aix-Marseille Université
347 PUBLICATIONS **3,899** CITATIONS
SEE PROFILE

Isabelle Bombarda
Aix-Marseille Université
60 PUBLICATIONS **391** CITATIONS
SEE PROFILE

Long-Chain Aliphatic β-Diketones from Epicuticular Wax of *Vanilla* Bean Species. Synthesis of Nervonoylacetone

Béatrice Ramaroson-Raonizafinimanana,† Emile M. Gaydou,* and Isabelle Bombarda

Laboratoire de Phytochimie de Marseille, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille III, avenue Escadrille Normandie Niemen, 13397 Marseille Cedex 20, France

Analysis of the neutral lipids from *Vanilla fragrans* and *Vanilla tahitensis* (Orchidaceae) without saponification resulted in the isolation and identification of a new product family in this plant: β-dicarbonyl compounds. The compound structures are composed of a long aliphatic chain with 2,4-dicarbonyl carbons and a cis double bond at the *n*−9 position. They represent ∼28% of the neutral lipids, that is, 1.5%, in immature beans, and ∼10% of the neutral lipids, that is, 0.9%, in mature beans. Using retention indices, gas chromatography−mass spectrometry, derivatization reactions, and chemical degradation, five β-dicarbonyl compounds have been identified including 16-pentacosene-2,4-dione, 18-heptacosene-2,4-dione, 20-nonacosene-2,4-dione, 22-hentriacontene-2,4-dione, and 24-tritriacontene-2,4-dione. Among them (*Z*)-18-heptacosene-2,4-dione, or nervonoylacetone, has been synthesized in two steps starting from nervonic acid. The major constituent, nervonoylacetone, represented 74.5% of the β-dicarbonyl fraction. The range of these compounds has been studied in relation with bean maturity for *V. fragrans* and *V. tahitensis* species. This compound family has not been found in the leaves or stems of any of the three vanilla species studied and is markedly absent in the beans of *V. madagascariensis*.

Keywords: *Vanilla beans; V. fragrans; V. tahitensis; V. madagascariensis; methyl ketones; fatty acids; β-diketones; long-chain fatty acids; trimethylsilyl derivatives; nervonoylacetone; GC-MS*

INTRODUCTION

Chemical examination of the neutral lipids from *Vanilla fragrans* before saponification revealed the presence of two polar fractions. The most polar one was recently identified as a new product family in these species: long-chain γ-pyrone compounds, which were characterized as 2,6-disubstituted-2,3-dihydropyran-4-ones (Ramaroson-Raonizafinimanana et al., 1999). We report here the isolation of new and unusual β-dicarbonyl compounds and the synthesis of one of them, called nervonoylacetone.

β-Diketones are relatively common constituents of plant waxes (Tulloch, 1976). They have been characterized in the leaf waxes from different Gramineae (Tulloch and Weenink, 1969; Tulloch and Hoffman, 1974, 1976; Tulloch, 1973, 1985), eucalyptus (Horn et al., 1964; Osawa and Namiki, 1985), some leguminous plants (Horn and Lamberton, 1962; Horn et al., 1964), and rhododendrons (Evans et al., 1975). Because this compound's family has not been characterized in the *Vanilla* genus, we have begun the research of this compound family in three *Vanilla* bean species: *V. fragrans*, *V. tahitensis*, and *V. madagascariensis*. The last species, *V. madagascariensis*, which is characterized by the absence of aroma and flavor, does not contain β-diketones.

* Corresponding author (telephone/fax 33 4 91 28 86 47; e-mail emile.gaydou@iut-chimie.u-3mrs.fr).

† Present address: Département Industries Agricoles et Alimentaires, Ecole Supérieure des Sciences Agronomiques, Université d'Antananarivo, Madagascar.

MATERIALS AND METHODS

***Vanilla* Bean Materials.** *V. madagascariensis*, a native species from Madagascar (1 sample), was collected in the Antalaha area (northeastern Madagascar). *V. fragrans* (12 samples) was collected in various areas (Comoros, Madagascar, Hawaii, and Indonesia), and *V. tahitensis* (4 samples) was collected in Tahiti. Green vanilla beans were collected at two stages of maturation. Mature beans correspond to the ninth month after pollination and immature beans to the seventh month after pollination. Vanilla beans are subjected to drying and curing processes during which their characteristic aroma and flavor are developed.

Extraction of β-Diketones. Beans were crushed (40 g) and extracted with pentane (150 mL) at reflux using a Soxhlet during 16 h to give a lipidic extract (8.6−14% from cured or 2−4% from green beans). Thin-layer chromatography (TLC) of the lipid extract using *n*-hexane/diethyl ether (90:10 v/v) and phosphomolybdic acid as revelator revealed an intense spot (R_f = 0.51−0.61) for *V. fragrans* and *V. tahitensis* different from triglycerides (R_f = 0.19−0.34) and hydrocarbons (R_f = 0.95−0.98) (Ramaroson-Raonizafinimanana et al., 1999). This extract (0.4 g) was fractionated using column chromatography (25 cm, i.d. = 2.4 cm, 50 g of SiO_2) with *n*-hexane/diethyl ether (90:10 v/v, 200 mL) and collected in eight tubes. Tubes 6−8 contained β-diketones (0.112 g, 28%).

Purification of the β-Diketone Fraction. Purification of the β-diketone fraction from lipidic extract (2.5 g) was achieved with 70 mL of hot *n*-hexane in 50 mL of saturated aqueous cuprous acetate (Horn et al., 1964). The cuprous β-dicarbonylated complex precipitated, and it was filtered and washed with ethanol (20 mL). A colorless oil was recovered by decomposition of the complex with 10 mL of 2 N hydrochloric acid. β-Dicarbonyl compounds were extracted with diethyl ether (3 × 15 mL). The organic layer was washed with $NaHCO_3$ and dried with Na_2SO_4.

10.1021/jf0002701 CCC: $19.00
Published on Web 09/13/2000

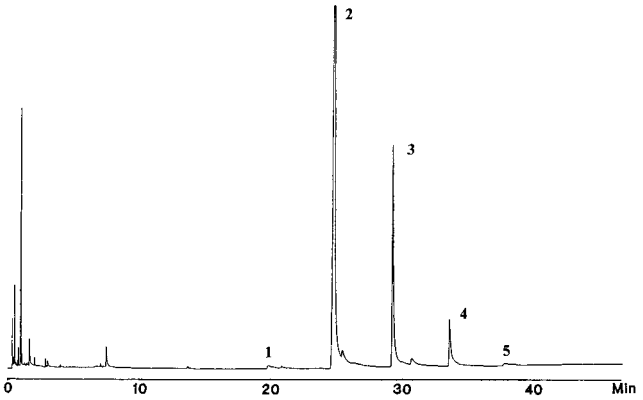


Figure 1. Gas chromatogram of the β-diketone fraction obtained by GC on an OV-1 column from 160 to 280 °C, 3 °C min^{-1}.

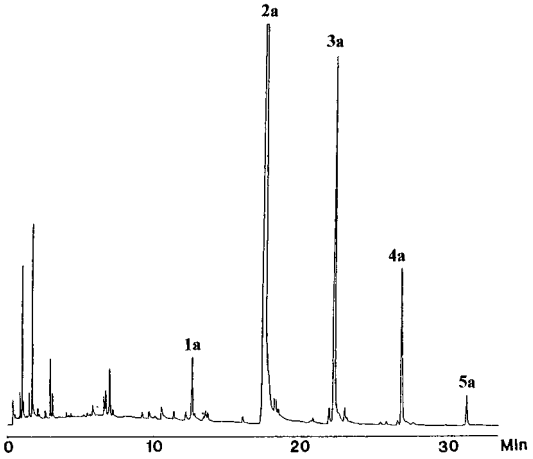


Figure 2. Gas chromatogram of the methyl ketone fraction obtained by GC on an OV-1 column from 160 to 280 °C, 3 °C min^{-1}.

Decomposition of β-Diketones. β-Diketones (0.5 g) were refluxed in 100 mL of ethanolic KOH (4% w/v) during 18 h. The saponified mixture (Scheme 1) was diluted with water (200 mL). The organic layer was washed with water and dried with Na_2SO_4 and then concentrated under reduced pressure to obtain an oil, which was purified by flash chromatography with *n*-hexane yielding the corresponding methyl ketones, **1a**−**5a** (Figure 2) (0.4 g). The remaining saponified mixture was acidified with 2 N HCl (20 mL), and the corresponding unsaturated fatty acids, **1b**−**5b** (Figure 3), extracted with

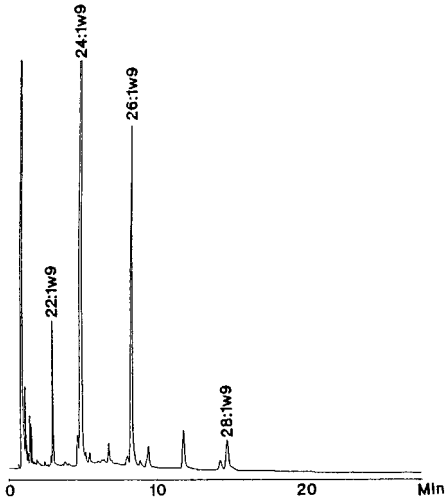


Figure 3. Gas chromatogram of the fatty acid fraction obtained by GC on Carbowax 20M column at 200 °C.

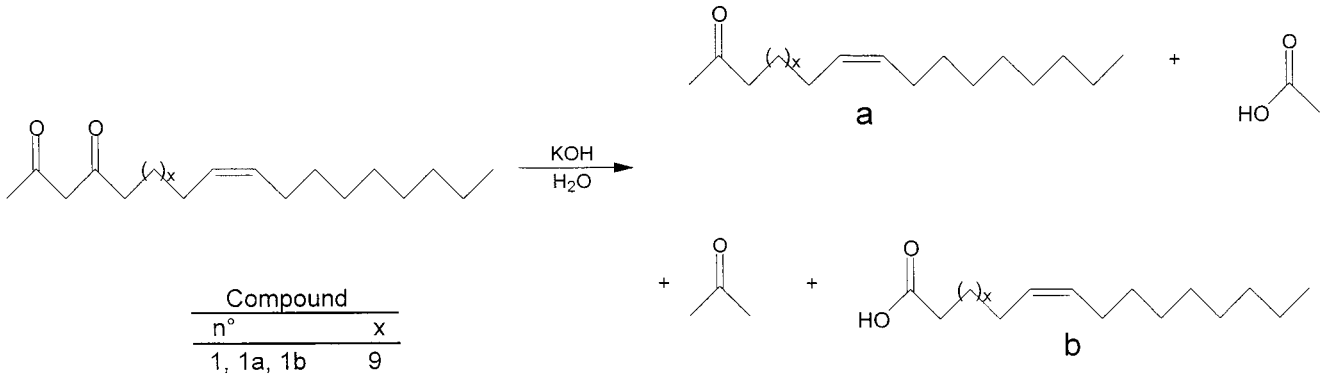
n-hexane (3 × 50 mL). The organic layer was washed, dried, and esterified as fatty acid methyl esters (FAME) using methanol and HCl as catalyst for GC analyses.

Derivatization Reactions. The dihydroxy-2,4-diones were obtained by oxidation of the double bond with OsO_4 in anhydrous dioxane according to the procedure of Mallet et al. (1985). The trimethylsilyl ether derivatives were prepared from glycol by reaction with a solution of bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% of trimethylchlorosilane (TMCS) (Mallet et al., 1985) (Scheme 2).

Synthesis of Nervonoylacetone. Sodium hydride (60% suspension in oil) (48 mg, 2 mmol) in 5 mL of anhydrous diethyl ether was stirred at room temperature with 247 mg of nervonic acid methyl ester (247 mg, 0.636 mmol). Anhydrous acetone (1.45 mL, 20 mmol) was slowly added with a syringe and the mixture maintained at 40−50 °C. The excess of sodium hydride was decomposed with 1 mL of ethanol. Crushed ice was poured into the reaction mixture, which was acidified with acetic acid. Nervonoylacetone was extracted with diethyl ether (3 × 15 mL), and the organic layer was washed with $NaHCO_3$ and dried over Na_2SO_4.

Gas Chromatography (GC). A Girdel 30 gas chromatograph equipped with a flame ionization detector (FID) was used for compound separations with an OV-1 fused silica capillary column (25 m × 0.32 mm i.d.) (phase thickness = 0.15 μm; column temperature = 160−280 °C, 3 °C min^{-1}). Detector and inlet temperatures were 300 and 295 °C, respectively. Hydrogen was used as a carrier gas at an inner pressure of 0.5 bar (3 mL/min, split = 60 mL/min). For FAME separa-

Scheme 1. Basic Hydrolysis of 2,4-Diketones 1−5 into Methylketones 1a−5a and Fatty Acids 1b−5b



Compound n°	x
1, 1a, 1b	9
2, 2a, 2b	11
3, 3a, 3b	13
4, 4a, 4b	15
5, 5a, 5b	17

Scheme 2. Formation of the 2,4-Diketone Silyl Ether Derivatives

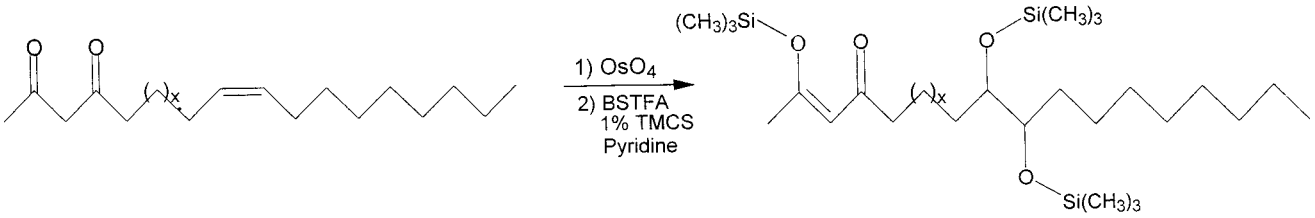


Table 1. Relative Composition of the β-Dicarbonyl Fraction, Methyl Ketone Fraction, and Fatty Acids

β-diketones			methyl ketones			fatty acids			
compd	I_R[a,b]	relative composition[a] (%)	compd	I_R[a,b]	relative composition[a] (%)	compd	structure	ECL[c]	relative composition[a] (%)
1	2758	tr[d]	**1a**	2476	2.3	**1b**	22:1ω9	22.20	3.6
2	2962	74.5	**2a**	2671	69.0	**2b**	24:1ω9	24.21	72.6
3	3166	20.0	**3a**	2868	20.1	**3b**	26:1ω9	26.18	15.4
4	3370	5.0	**4a**	3066	7.2	**4b**	28:1ω9	28.16	2.2
5	3569	tr	**5a**	3268	1.4	**5b**	30:1ω9	30:14	tr

[a] Determined on a 25 m capillary column (OV-1). [b] Retention indices. [c] Determined on a 25 m capillary column coated with Carbowax 20M. [d] Trace.

tions, a fused silica capillary column (25 m × 0.32 mm i.d.) coated with Carbowax 20M (phase thickness = 0.15 μm; column temperature = 200 °C) was used. Detector and inlet temperatures were 250 and 240 °C, respectively. The injections averaged 1 μL of a 0.5% solution of crude mixtures in hexane. For quantitative analysis, the injections averaged 1 μL of a solution prepared as followed: to 1 mL of the solution A [raw extract (200 mg) in hexane (100 mL), i.e., 2 mg/mL] was added 0.25 mL of the solution B [octacosane (25 mg) in hexane (100 mL), i.e., 0.25 mg/mL].

GC−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. × 350 mm, 10^{-6} mmHg) and coupled with a Sidar data computer. The GC column was an OV-1701 fused capillary column (50 m × 0.32 mm, 0.30 μm phase thickness). The column temperature was 100−280 °C, 3 °C min^{-1}; the carrier gas was helium (2 bar, 4 mL/min, split = 80 mL/min); the ion source temperature was 270 °C; and the ionizing voltage was 70 eV.

Spectroscopic Procedures. UV spectra were recorded with a Beckman UV DU-20 spectrophotometer. IR spectra were recorded with a Perkin-Elmer 457 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 200 MHz on a Bruker spectrometer. Tetramethylsilane (TMS) was used as internal standard in both measurements.

NMR Data of Nervonoylacetone ***2****:* ^1H NMR enolic tautomer, δ 0.82 (t, H-C_{27}), 1.19 (m, H-C_8 to H-C_{16} and H-C_{21} to H-C_{26}), 1.52 (m, H-C_{17} and H-C_{20}), 1.95 (t, H-C_5), 2.00 (s, H-C_{20}), 5.28 (t, H-C_{18} and H-C_{20}), 5.41 (s, H-C_3); ^1H NMR ketonic tautomer, δ 2.18 (s, H-C_1), 2.40 (t, H-C_5), 3.50 (s, H-C_3).

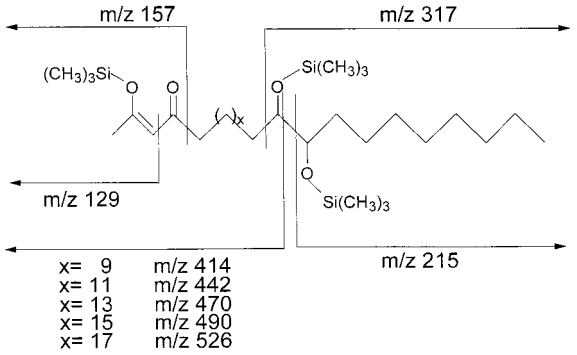
^{13}C NMR 14.1 (CH_3, C-27), 22.7 (CH_2, C-26), 24.9 (CH_3, C-1), 25.8 (CH_2, C-6), 32.0 (CH_2, C-17 and C-20), 38.3 (CH_2 enolic tautomer, C-5), 44.2 (CH_2 ketonic tautomer, C-5), 57.6 (CH_2 ketonic tautomer, C-3), 99.7 (CH enolic tautomer, C-3), 129.9 (CH, C-18 and C-19), 190.2 (C, C-2), 193.8 (C, C-4).

RESULTS AND DISCUSSION

The neutral lipid content of various *Vanilla* bean samples (*V. fragrans*, *V. tahitensis*, and *V. madagascariensis*) obtained by *n*-pentane extraction varied from 8.6 to 14.0% for cured beans and from 2.2 to 4.2% for green beans. The polar fraction was obtained from the neutral lipid fraction by liquid chromatography over silica. In the case of *V. madagascariensis*, TLC analysis did not reveal a spot corresponding to this fraction. GC analysis of this fraction showed five major peaks (Figure 1) showing the presence of at least five compounds (**1**−**5**), which represented 28.0% (mean value of four samples) of the neutral lipid fraction. Identification of compounds **1**−**5** was achieved by IR, UV, and GC-MS analyses and by reaction of degradation after purification of the β-diketone fraction using copper acetate (Horn and Lamberton, 1962).

Qualitative Analyses. The IR spectrum of the mixture of compounds **1**−**5** showed absorption at 1610 cm^{-1}, which is in agreement with the presence of the enol tautomer (Horn et al., 1964; Tulloch and Weenink, 1969; Trka and Streibl, 1974), and an absorption at 1725 cm^{-1} corresponding to the keto tautomer. The absence of a band at 967 cm^{-1}, generally characteristic of trans disubstituted double bonds in fatty acid chains, confirmed the presence of a cis configuration for the double bond usually observed in most natural fatty acids (Mallet et al., 1983). The UV spectrum showed an absorption at 274 nm, which is consistent with the presence of a β-dicarbonyl moiety. Purification of this fraction using aqueous cuprous acetate (Horn et al., 1964) allowed isolation of a colorless oil. The mass spectrum obtained using GC-MS of compounds **1**−**5** showed the presence of fragments at *m/z* 100 (85−100%) characteristic of β-dicarbonyl compounds (Trka and Streibl, 1974). Molecular peaks were observed at *m/z* 378 ($C_{25}H_{46}O_2$, 0.8% for compound **1**), 406 ($C_{27}H_{50}O_2$, 2.4% for compound **2**), 434 ($C_{29}H_{54}O_2$, 1.2% for compound **3**), 462 ($C_{31}H_{58}O_2$, 1.0% for compound **4**), and 490 ($C_{33}H_{62}O_2$, 0.5% for compound **5**). Furthermore, characteristic fragments of monounsaturated hydrocarbons were observed at *m/z* 69 (45.0−54.1%), 55 (72−82.5%), 43 (68.5−92.8%), and 41 (29.2−37.3%). Compounds **1**−**5** were therefore β-diketones having a cis disubstituted double bond and containing 25, 27, 29, 31, and 33 carbon atoms, respectively. To establish the positions of the β-dicarbonyl group and the double bond in the chain, we performed a hydrolysis in basic medium of the β-diketones using Tulloch and Weenink's (1969) procedure (Scheme 1). Five methyl ketones (**1a**−**5a**) were obtained (Figure 2). Retention indices and relative percentages of compounds **1a**−**5a** are given in Table 1. Mass spectra of these compounds showed the loss of acetone with the fragments at *m/z* 278 for **1a** (0.9%), *m/z* 306 for **2a** (2.5%), *m/z* 334 for **3a** (2.2%), *m/z* 362 for **4a** (0.7%), and *m/z* 390 for **5a** (0.7%). The fragment

Scheme 3. Characteristic Fragments of the 2,4-Diketone Silyl Ether Derivatives



at *m/z* 125 (25.1−35.0%) for all compounds **1a**−**5a** confirmed the *n*−9 position of the double bond. Furthermore, molecular peaks observed at *m/z* 336 M^+, $C_{23}H_{44}O$ (17%), *m/z* 364 M^+, $C_{25}H_{48}O$ (6.0%), *m/z* 392 M^+, $C_{27}H_{52}O$ (4.7%), *m/z* 420 M^+, $C_{29}H_{56}O$ (0.5), and *m/z* 448 M^+, $C_{31}H_{60}O$ (2.3%) were in agreement with methyl ketones **1a**−**5a** containing 23, 25, 27, 29, and 31 carbon atoms, respectively. Mass spectra of compounds **1a**−**5a** proved the 2,4-dione position of *β*-dicarbonyl moiety in the fatty chain of compounds **1**−**5**. To confirm the *n*−9 position of the cis double bond and the 2,4-position of the *β*-dicarbonyl group in **1**−**5**, we prepared the corresponding trimethylsilyl ether derivatives (Scheme 2). Reaction occurred with the double bond (Mallet et al., 1983, 1984, 1985; Dommes et al., 1976) and with the *β*-diketone moiety (Tulloch and Hogge, 1978). The fragment at *m/z* 157 (20.4%, Scheme 3) confirmed the 2,4-position of the dicarbonyl group for compounds **1**−**5**. The fragment at *m/z* 157 (20.4%) was in agreement with the 2,4-position of the dicarbonyl group for compound **1**. The 2-position of the silyl group is confirmed by the presence of a fragment *m/z* 129 (32.0%) resulting from the loss of a CO by the fragment *m/z* 157. The *n*−9 position of the double bond was established by the fragment at *m/z* 215 (100%), the corresponding trimethylsilyl ether derivative $(CH_3)_3SiOCH(CH_2)_7CH_3]^+$ (Mallet et al., 1983, 1984, 1985; Dommes et al., 1976; Tulloch and Hogge, 1978) and by fragments at *m/z* 442 (7.2%) and *m/z* 317 (0.9%) corresponding to $(CH_3)_3$-$SiOCHCH(OSi(CH_3)_3)(CH_2)_7CH_3]^+$. The ^1H NMR spectrum of **2** was relatively complex because of the occurrence of the keto enolic equilibrium (Gilli et al., 1989) and showed chemical shifts in agreement with those observed by Nonhebel et al. (1968) for acetyl ketone. The ^{13}C NMR spectrum showed chemical shifts for keto enolic carbons which were in concordance with those proposed by Tulloch (1985) for pentacosane-8,10-dione (^1H and ^{13}C NMR data are given under Materials and Methods).

In the course of the *β*-diketone degradation in basic medium, we obtained also five fatty acids, including erucic (**1b**), nervonic (**2b**), ximenic (**3b**), *cis*-octacos-9-enoic (**4b**), *cis*-triacont-9-enoic acid (**5b**), lignoceric acid, and three unidentified other compounds (Figure 3). Relative percentages and equivalent chain length (ECL) on Carbowax 20M are given in Table 1. Fatty acid identifications was achieved using ECL, by GC-MS, and by comparison with an authentic sample for nervonic acid (**2b**), which constituted 72.6% of the total products. Ximenic acid (**3b**) represented 15.4%. The other acids were minor products.

Table 2. *β*-Diketone Composition[a] of *V. fragrans* Cured Beans from Different Origins and at Two Stages of Maturity

	Comoros	Madagascar		Hawaii	Indonesia
compd	immature[b]	immature[b]	mature[c]	mature[c]	mature[c]
2	15.2	12.0	10.3	5.8	4.8
3	4.5	4.1	3.4	2.4	1.5
4	1.9	1.6	1.4	0.9	0.5
5	0.3	0.5	0.2	tr[e]	tr
Σ**2**−**5**[d]	21.9	18.2	15.3	9.1	6.8

[a] Weight percentage based on lipidic extract and determined on OV-1 capillary columns. [b] Beans were collected during the seventh month after pollination. [c] Mature beans were collected during the ninth month after pollination. [d] Sum of the *β*-diketone percentages. [e] Trace.

Table 3. *β*-Diketone Composition[a] of *V. fragrans* from Madagascar and *V. tahitensis* from Tahiti Green Beans at Two Stages of Maturity

	V. fragrans		*V. tahitensis*	
compd	immature[b]	mature[c]	immature[b]	mature[c]
2	16.1	9.5	8.3	7.9
3	6.2	2.9	2.8	2.2
4	2.6	1.2	1.4	1.1
5	0.4	tr[e]	0.3	0.1
Σ**2**−**5**[d]	25.3	13.6	12.8	11.3

[a] Weight percentage based on lipidic extract and determined on OV-1 capillary columns. [b] Beans were collected during the seventh month after pollination. [c] Mature beans were collected during the ninth month after pollination. [d] Sum of the *β*-diketone percentages. [e] Trace.

Nervonoylacetone (**2**) was synthesized from nervonic acid methyl ester using sodium hydride. Such a Claisen acylation method was used with success in the synthesis of *β*-diketones from saturated fatty acids (Trka and Streibl, 1974; Adams and Hauser, 1944; Swamer and Hauser, 1950). Spectral data of synthesized nervonoylacetone were in agreement with spectroscopic data of the natural product **2**.

Quantitative Analysis. The *β*-dicarbonyl compound fraction was found to contain 16-pentacosene-2,4-dione (**1**), 18-heptacosene-2,4-dione (**2**), 20-nonacosene-2,4-dione (**3**), 22-hentriacontene-2,4-dione (**4**), and 24-tritriacontene-2,4-dione (**5**). The relative composition of this fraction and retention indices on OV-1 of compounds **1**−**5** are given in Table 1. Nervonoylacetone (**2**) was the major component and represented 74.5% of the *β*-dicarbonyl fraction, whereas compounds **1** and **5** were found in lower concentrations. Compounds **3** and **4** represented 20.0 and 5.5% of this fraction, respectively. A quantitative study of the *β*-diketone compounds was achieved by GC analysis with octacosane as internal standard (I_R = 2800). We first studied the percentage response of this compound family in the lipidic fraction from *V. fragrans* cured beans of different origins at two stages of maturity. Results are summarized in Table 2. For all samples, compound **2** was the major one (4.8−15.2%). Mature beans from Indonesia and Hawaii were characterized by the lowest contents of **2** (4.8 and 5.8%, respectively). For Madagascar beans, the *β*-diketone percentages were higher in immature beans than in mature beans. This result was confirmed when the lipidic fractions from green beans (mature and immature) were investigated. The results from green *V. fragrans* (Madagascar) and *V. tahitensis* (Tahiti) beans are given in Table 3. As in the case of cured beans,

compound **2** was the major β-diketone in green beans with 9.5−16.9% and 7.9−8.3%, respectively. The percentage of β-diketones was higher in immature beans than in mature beans. The decreasing percentage of β-diketones with bean development observed in green and cured beans showed that curing and drying processes have little influence on the β-diketone relative contents and percentages in lipidic vanilla bean extract.

This compound family has not been characterized in the leaves or stem of *Vanilla* beans. Such compounds have not been characterized in *V. madagascariensis*, the beans of which are odorless. Therefore, the 2,4-diketone family seems to be specific to the lipidic fraction of the two species (*V. fragrans* and *V. tahitensis*) harvested for their vanilla aroma.

ACKNOWLEDGMENT

We are grateful to M. Derbesy for providing authentic *Vanilla* bean samples from Comoros, Hawaii, Indonesia, and Tahiti.

LITERATURE CITED

Adams, J. T.; Hauser, C. R. The acylation of methylketones with aliphatic esters by means of sodium amide. Synthesis of β-diketones of the type $RCOCH_2COR$. *J. Am. Chem. Soc.* **1944**, *66*, 1220−1222.

Dommes, V.; Wirtz-Peitz, F.; Kunau, W. H. Structure determination of polyunsaturated fatty acids by gas chromatography-mas spectrometry. A comparison of fragmentation patterns of various derivatives. *J. Chromatogr. Sci.* **1976**, *14*, 360.

Evans, D.; Knights, B. A.; Math, V. B.; Ritchie, A. L. β-Diketones in *Rhododendron* waxes. *Phytochemistry* **1975**, *14*, 2447−2451.

Gilli, G.; Belluci, F.; Ferretti, V.; Bertolasi, V. Evidence for resonance-assisted hydrogen bonding from crystal-structure correlations on the enol form of the β-diketon fragment. *J. Am. Chem. Soc.* **1989**, *111*, 1023−1028.

Horn, D. H. S.; Lamberton, J. A. Long chain β-diketones from plant waxes. *Chem. Ind.* **1962**, *1*, 2036−2037.

Horn, D. H. S.; Kranz, Z. H.; Lamberton, J. A. Composition of *Eucalyptus* and some other leaf waxes. *Aust. J. Chem.* **1964**, *17*, 464−476.

Madison, B. L.; Depalma, R. A.; D'Alonzo, R. P. Accurate determination of *trans* isomers in shortings and edible oils by infrared spectrophotometry. *J. Am. Oil Chem. Soc.* **1982**, *59*, 178−181.

Mallet, G.; Morin, O.; Ucciani, E. Hydrogenation of trienic fatty acids. I. The *cis* isomers obtained by hydrogenation of α- and γ-linolenic acids by hydrazine. *Rev. Fr. Corps Gras* **1983**, *30*, 453−462.

Mallet, G.; Ucciani, E.; Morin, O. Hydrogenation of trienic fatty acids. II. *Trans* isomers obtained by isomerization of products from the hydrogenation of α- and γ-linolenic acids by hydrazine. *Rev. Fr. Corps Gras* **1984**, *31*, 347−355.

Mallet, G.; Dimitriades, C.; Ucciani, E. Study on derivatization techniques for the separation of fatty acid esters by capillary gas chromatography. *Rev. Fr. Corps Gras* **1985**, *32*, 439−446.

Mikkelsen, J. D. Structure and biosynthesis of β-diketones in barley spike epicuticular wax. *Carlsberg Res. Commun.* **1979**, *44*, 133−147.

Nonhebel, D. C. NMR spectra of intramolecularly hydrogen-bonded compounds—I. β-diketones, *o*-hydroxyaldehydes, and *o*-hydroxyketones. *Tetrahedron* **1968**, *24*, 1869−1894.

Osawa, T.; Namiki, M. Natural oxidants isolated from *Eucalyptus* leaf waxes. *J. Agric. Food Chem.* **1985**, *33*, 777−780.

Ramaroson-Raonizafinimanana, B.; Gaydou, E. M.; Bombarda, I. Hydrocarbons from three Vanilla bean species: *V. fragrans*, *V. madagascariensis* and *V. tahitensis*. *J. Agric. Food Chem.* **1997**, *45*, 2542−2545.

Ramaroson-Raonizafinimanana, B.; Gaydou, E. M.; Bombarda, I. Long-chain γ-pyrones in epicuticular wax of two vanilla bean species: *V. fragrans* and *V. tahitensis*. *J. Agric. Food Chem.* **1999**, *47*, 3202−3205.

Swamer, F. W.; Hauser, C. R. Claisen acylations and carbethoxylations of ketones and esters by means of sodium hydride. *J. Am. Chem. Soc.* **1950**, *72*, 1352.

Tulloch, A. P. Composition of leaf surface waxes of *Triticum* species. Variation with age and tissue. *Phytochemistry* **1973**, *12*, 2225−2232.

Tulloch, A. P. In *Chemistry and Biochemistry of Natural Waxes*; Kolattukkudy, P. E., Ed.; Elsevier: Amsterdam, The Netherlands, 1976; p 235.

Tulloch, A. P. Carbon-13 NMR spectra of β-diketones from wax of the gramineae. *Phytochemistry* **1985**, *24*, 131−137.

Tulloch, A. P.; Hoffman, L. L. Epicuticular waxes of secale cereal and tricale hexaploide leaves. *Phytochemistry* **1974**, *13*, 2535−2540.

Tulloch, A. P.; Hoffman, L. L. Epicuticular wax of *Apropyron intermedium*. *Phytochemistry* **1976**, *15*, 1145−1151.

Tulloch, A. P.; Hogge, L. R. Gas chromatographic-mass spectrometric analysis of β-diketone-containing plant waxes. Use of trimethylsilyl ethers. *J. Chromatogr.* **1978**, *157*, 291−296.

Trka, A.; Streibl, M. Mass spectra of long-chain aliphatic β-diketones and some of their derivatives. *Collect. Czech. Chem. Commun.* **1974**, *39*, 468−474.

Tulloch, A. P.; Weenink, R. O. Composition of the leaf wax of little club wheat. *Can. J. Chem.* **1969**, *47*, 3119−3126.

Received for review March 2, 2000. Revised manuscript received June 15, 2000. Accepted June 16, 2000.

JF0002701