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HPLC AND GC-MS IDENTIFICATION OF THE MAJOR ORGANIC CONSTITUENTS IN NEW ZEALAND PROPOLIS

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Key Word Index—New Zealand propolis; HPLC; GC-MS; flavonoids; aromatic substances; fatty acids; 5-phenyl-*trans*-3-pentenoic acid.

Abstract—The major organic constituents of New Zealand sourced propolis have been identified and quantified in tincture solutions by a combination of HPLC and GC-mass spectrometry (MS). HPLC was the preferred method for the analysis of flavonoids because of their very low response factors in GC-MS. Flavonoid levels ranged from 30–40 mg ml^{-1}. A distinctive characteristic of the flavonoids in New Zealand propolis is the unusually high proportion (*ca* 70%) of dihydroflavonoids, e.g. pinocembrin, pinobanksin and pinobanksin 3-acetate. Non-flavonoid components analysed by GC-MS comprised a range of aromatic compounds (3–7.5 mg ml^{-1}), together with low levels (0.25–0.78 mg ml^{-1}) of fatty acids. The former comprised mainly cinnamic acids and their esters, but also included the rare 5-phenyl-*trans-trans*-2,4-pentadienoic acid and the new natural product, 5-phenyl-*trans*-3-pentenoic acid. Both were synthesized to confirm their identity.

INTRODUCTION

Propolis, or 'bee glue', is a complex resinous mixture of plant-derived products gathered, modified and used by bees as a general purpose sealer, draught excluder and antibiotic in their hives. Propolis typically consists of waxes, resins, water, inorganics, phenolics and essential oils [1], the exact composition of which is dependent upon the source plant(s). Propolis balsam, or tincture, is an ethanolic extract of raw (natural) propolis containing the bulk of the organic constituents [2]. This organic fraction is commonly incorporated into medicinal and healthfood products. Such extracts and their components have been shown to exhibit antibacterial, antifungal, antiviral and antioxidative activities [1, 3–7].

In Europe, North and South America and western Asia the dominant propolis source is the bud exudate of poplar (*Populus*) [8]. Less commonly, in other parts of the world, species such as *Betula* (birch), *Ulmus* (elms), *Pinus* (pines), *Quercus* (oaks), *Salix* (willow) and *Acacia* (wattle) are utilized as propolis sources [1, 8, 9]. The geographical dependence of propolis constituents is exemplified in analyses of, e.g. European, South American, Chinese, Canadian and Spanish sourced samples [9–13]. Very little work has been carried out on the quantification of individual propolis components [14].

New Zealand has a unique native flora resulting from long geographic isolation, together with introduced known about the composition of New Zealand produced propolis, a significant proportion of which is currently being utilized in commercial products derived from tinctures. We now report the results of a quantitative HPLC and GC-mass spectrometric investigation of a series of New Zealand produced propolis tinctures, derived from propolis collected from hives located in a variety of geographic regions.

RESULTS AND DISCUSSION

Flavonoid constituents

Qualitative and quantitative comparisons of the flavonoid profiles of propolis from a variety of geographic regions in New Zealand were carried out in order to assess any variations due to catchment area. Flavonoid constituents were quantified by HPLC on a C-18 reverse phase column. The absorption spectra resulting from diode-array detection were used to distinguish peaks due to the major flavonoids from those of other UV absorbing components, predominantly cinnamic acid derivatives. Flavonoid identification was carried out by direct HPLC comparison with authentic standards and was based on co-chromatography in two solvents and on the identity of the absorption spectra. Additionally, many identifications were also confirmed by GC-mass spectrometry (GC-

Table 1. HPLC determined flavonoid (and other) constituents of New Zealand propolis tincture solutions

			Samples							
Constituent	Rt_A	Rt_B	20/1 (22.5%)*	20/2 (19.9%)*	20/3 (17.0%)*	20/4 (20.9%)*	20/5 (18.7%)*	20/6 (20.5%)*	20/7 (16.7%)*	20/8 (19.3%)*
Cinnamic acid	10.51		p	p	p	p	p	p	p	p
Pinobanksin†	11.18	14.50	3.8	3.2	3.5	3.0	3.4	3.2	3.6	2.2
Pinocembrin†	24.51		10.8	8.9	8.1	11.2	9.1	8.8	9.0	6.7
Pinobanksin 3-acetate†	27.85	24.58	9.4	7.5	7.2	7.2	7.7	8.0	6.3	8.7
1,1-Dimethylallylcaffeic acid	28.60		p	p	p	p	p	p	p	p
Chrysin‡	31.33		5.0	4.8	4.3	3.9	4.9	4.3	4.3	4.1
Galangin‡	33.65		3.8	3.8	3.2	2.8	3.6	3.5	3.3	2.8
Pinocembrin 7-methyl ether†	41.40		2.0	1.9	2.0	1.8	1.9	1.9	1.5	2.0
Chrysin 7-methyl ether‡	45.05		0.6	0.7	0.6	0.4	0.7	0.4	0.4	0.7
Galangin 7-methyl ether‡	47.10		0.4	0.4	0.4	0.3	0.5	0.4	0.2	0.4
Internal standard	49.10									
Total flavonoids¶			35.8	31.2	29.3	30.6	31.8	30.5	28.6	27.6

Subscripts A and B = solvent systems A and B, respectively.

p = Constituent present, but not quantified.

*Dissolved solids as % (by wt) of tincture.

†Levels calculated as naringenin equivalents (mg mL^{-1} tincture).

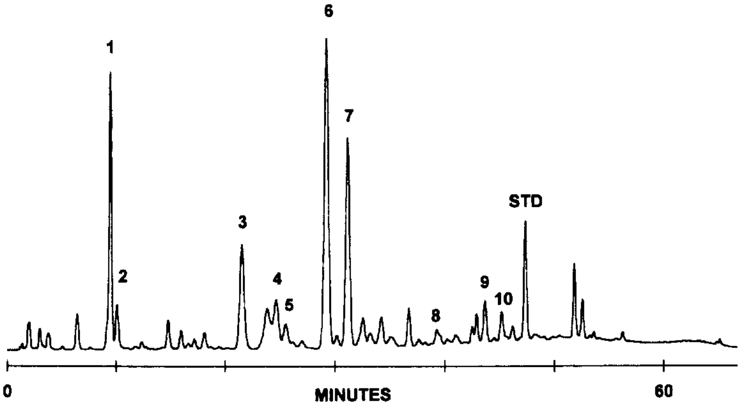
‡Levels calculated as chrysin equivalents (mg ml^{-1} tincture).

¶Non-identified minor flavonoids increase these figures by *ca* 10%.

because it is a common propolis constituent and has been implicated as a causative agent of contact allergies in bee keepers [15].

One other cinnamic acid, cinnamic acid itself, is a major component in the HPLC trace (peak 1, Fig. 1) and this was identified and quantified by GC-MS (see below). The remaining minor HPLC peaks were not identified, but were shown to represent flavonoids and cinnamic acid derivatives on the basis of their absorption spectra. Thus, minor flavonoids, which combined represented less than 10% of the total flavonoids, were identified with Rt_A values of 13.2, 15.9, 17.1, 18.7, 34.8, 43.0, 44.7 and 48.1. The remaining minor components possessed cinnamic acid-type absorption spectra and were subsequently identified by GC-MS (see below).

To achieve sufficient resolution of all major components for quantification it was necessary to carry out HPLC analysis in two different solvent systems, A and B (see Experimental). Solvent system B was of particular value in the resolution of pinobanksin and pinobanksin 3-acetate. Quantification was achieved using synthetic 5,7-dimethyl-4'-methoxyflavanone as internal standard. Response factors for chrysin and naringenin relative to this internal standard were determined in solvent systems A and B and used to



calculate the levels of flavones/flavonols as chrysin equivalents, and dihydroflavonoids as naringenin equivalents. Absorption spectra recorded for all peaks confirm that the flavonoids in each group have band II absorptions at wavelengths similar to these reference compounds.

It is clear that pinocembrin and pinobanksin 3-acetate are the dominant flavonoids in New Zealand propolis (see Table 1). This is true for samples from all geographic regions sampled. A further feature is the lack of significant variation between regions. Qualitatively, New Zealand sourced propolis flavonoids resemble those found in propolis from other temperate regions such as Europe and North America [9]. As such, it can be inferred that New Zealand propolis originates predominantly from introduced species of the type found in Europe and North America (e.g. poplar [2]), although New Zealand native plant sources cannot be ruled out without further investigation. For example, species of the genus *Cassinia* (Compositae), which include the widespread endemic cottonwood (*C. leptophylla*), are known to deposit pinocembrin, pinobanksin and pinobanksin 3-acetate as major components in their leaf exudates [16] which could be collected by bees.

Dihydroflavonoids based on pinobanksin and pinocembrin comprise *ca* 70% of the flavonoids in the samples analysed, and this predominance of dihydroflavonoids appears to be a feature of this New Zealand propolis. For example, in one of the few recently available quantitative studies of propolis phenolics [12], one Brazilian, six Uruguayan and eight Chinese sourced samples were analysed by HPLC. Dihydroflavonoids were shown to comprise less than 10% of the total flavonoids in all but the Brazilian sample, which alone approached the 50% level. The predominant flavonoids in the Chinese and Uruguayan samples were flavones and flavonols.

The flavonoids in propolis are thought to account for much of the biological activity [6, 17], and in this respect it is of interest to note that dihydroflavonoids have been shown recently to have vitamin C sparing activity [18] and antihyperlipidemic activity [19] in addition to the earlier established antimicrobial activity [14].

Non-flavonoid components

Samples of the methylated ethanolic tincture solutions were also analysed by combined GC-MS. Comparison of the GC-MS profiles determined for ethylated and methylated tincture solutions established that the principle non-flavonoid components of the tincture solutions were an array of free and ethylated aliphatic and aromatic acids, together with significant levels of ferulic acid and some methylbutenyl ferulic acid esters. of the dominant aromatic and aliphatic substances identified in the tincture solutions are given in Table 2. Mass spectral data for the corresponding methylated derivatives are given in the Experimental section.

Analyses of the GC-MS profiles determined for the ethylated and methylated tincture solutions demonstrated that in some cases methylation (or ethylation) of phenolic hydroxyl groups had occurred during the derivatization process. For example, vanillin (4-hydroxy-3-methoxybenzaldehyde) was detected as 3,4-dimethoxybenzaldehyde in extracts derivatized with diazomethane, whereas diazoethane derivatization afforded 3-methoxy-4-ethoxybenzaldehyde. On the other hand, some of the aliphatic and aromatic acids were found to be 'naturally' present in the ethanolic tincture solutions as the corresponding ethyl esters. The presence of ethylated aliphatic and aromatic acids in the tincture solutions can be attributed to the progressive reaction of the parent acids with the ethanol during the shelf life of the tincture solutions. It is notable that the tendency towards ethyl ester formation was greater for fatty acids than was the case for aromatic acids or flavonoids.

The tendency for acidic substances progressively to form ethyl esters inhibited the use of an aliphatic or an aromatic acid (e.g. heptadecanoic acid or 4-methoxybenzoic acid) as primary quantification standards. An alternative approach was therefore adopted of adding a chemically unreactive *n*-alkane (*n*-octadecane) as the primary quantification standard and determining the response factors of the principal aromatic and aliphatic acids (cinnamic acid and palmitic acid, respectively) relative to this. The GC-MS response factor (R_f) for chrysin was also determined. Somewhat unexpectedly a R_f value of 0.03 was obtained, indicating that the GC-MS ion current arising from flavonoids is low compared to that arising from aromatic and aliphatic acids (0.51 and 0.94). For this reason, HPLC is considered to be the preferred method for flavonoid analyses. Pilot scale methylation reactions demonstrated that the 7-hydroxyl group of flavonoids such as chrysin reacted with diazomethane to afford the corresponding methyl ether. However, the hydrogen bonded 5-hydroxyl group was comparatively resistant towards methylation, although on occasions traces of 5,7-dimethylation were observed. GC-MS analysis (see Experimental) confirmed the presence in the methylated tincture solutions of the flavonoid substances (usually as the corresponding 7-methyl ethers) identified in HPLC analyses.

Two phenylpentenoic acids, namely 5-phenyl-*trans*-3-pentenoic acid and 5-phenyl-*trans-trans*-penta-2,4-dienoic acid, were identified in the tincture solutions (as the corresponding methyl or ethyl esters). The latter acid has previously been identified in propolis samples by Greenaway *et al.* [2]; however, a computer assisted search of the *Chemical Abstracts* database indicated the

Table 2. GC-MS determined concentrations (mg ml^{-1}) of aromatic and aliphatic compounds identified in propolis tincture solutions

	Sample number							
Compound	20/1 (22.5%)*	20/2 (19.9%)*	20/3 (17.0%)*	20/4 (20.9%)*	20/5 (18.7%)*	20/6 (20.5%)*	20/7 (16.7%)*	20/8 (19.3%)*
Aromatic compounds								
Styrene	0.05	0.04	0.08	0.03	0.04	0.02	0.03	nd
Benzyl alcohol	0.04	0.06	0.02	0.02	0.04	0.04	0.05	0.02
Benzoic acid†	0.63	0.45	0.25	0.20	0.35	0.42	0.17	0.37
2-Phenylethanol	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.01
3-Phenylpropanoic acid†	0.21	0.14	0.10	0.22	0.10	0.14	0.06	0.13
Ethyl 3-phenylpropanoate	0.06	0.05	0.03	0.09	0.06	0.04	0.03	0.03
Cinnamic acid†	2.72	2.00	1.40	3.89	2.15	2.19	0.91	1.90
Vanillin†	nd	0.05	0.03	0.03	0.05	0.05	0.03	0.04
Ethyl cinnamate	0.06	0.05	0.05	0.10	0.08	0.04	0.07	0.04
5-phenyl-*trans*-3-pentenoic acid	0.12	0.22	0.12	0.19	0.17	0.17	0.09	0.14
Ethyl 5-phenyl-*trans*-3-pentenoate	0.06	0.15	0.07	0.09	0.11	0.10	0.06	0.05
Vanillic acid†	0.01	0.05	0.04	0.03	0.05	0.03	0.05	0.02
p-Coumaric acid†	0.08	0.33	0.18	0.16	0.20	0.23	0.13	0.24
5-Phenyl-*trans*-*trans*-2,4-pentadienoic acid†	0.27	0.55	0.40	0.45	0.49	0.54	0.36	0.44
Ferulic acid†	nd	0.03	0.12	0.22	0.19	0.20	0.13	0.15
Ethyl ferulate	0.39	1.05	0.77	0.95	0.85	0.80	0.58	0.74
3-Methylbut-2-enyl ferulate	0.12	0.58	0.39	0.60	0.46	0.39	0.25	0.39
3-Methylbut-3-enyl ferulate	0.05	0.36	0.23	0.24	0.02	0.20	0.15	0.20
Total aromatic compounds	4.90	6.18	4.30	7.53	5.43	5.62	3.17	4.91
Fatty acids								
Myristic acid (14:0)	nd	nd	0.01	nd	nd	0.01	nd	0.01
Ethyl myristate	nd	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Palmitic acid (16:0)	0.04	0.06	0.09	0.06	0.06	0.08	0.08	0.06
Ethyl palmitate	0.07	0.10	0.15	0.10	0.13	0.10	0.16	0.07
Linoleic acid (18:2)	nd	0.04	0.04	0.05	0.04	0.04	0.03	0.03
Oleic acid (18:1)	0.05	0.12	0.15	0.10	0.11	0.13	0.11	0.09
Stearic acid (18:0)	tr	0.01	0.02	0.01	0.01	0.02	0.01	0.01
Ethyl linoleate	0.01	0.04	0.03	0.04	0.05	0.03	0.05	0.02
Ethyl oleate	0.07	0.15	0.25	0.15	0.23	0.13	0.21	0.09
Ethyl stearate	0.01	0.02	0.02	0.02	0.03	0.02	0.02	0.01
Total fatty acids	0.25	0.55	0.77	0.54	0.67	0.57	0.68	0.40

ssolved solids as % (by wt) of tincture.

tected as the corresponding methyl ester and/or phenol methyl ether (see Experimental).

= not detected; tr = trace (<0.005 mg/mL).

authentic samples which were synthesized for this purpose (see Experimental).

The levels of aromatic acids identified in this study can be compared to those reported elsewhere. Chi *et al.* [20] reported levels of 3,4-dimethoxycinnamic acid (3.84 mg g^{-1}), isoferulic acid (3.33 mg g^{-1}), quercetin (0.25 mg g^{-1}) and caffeic acid (2.34 mg g^{-1}) in other propolis samples. Generally, similar levels have been reported in Chinese and South American samples [12]. In addition to the compounds listed in Table 2, GC-MS analysis demonstrated the presence of moderate levels of five oxygenated sesquiterpenes, each of which exhibited strong m/z 59 fragment ions attributable to the presence of a hydroxy-isopropyl group [$-CH(OH)(CH_3)_2$]. One of these compounds was identified as α-eudesmol, by comparison with the GC-MS characteristics of a specimen of this compound available in our laboratory. The levels of these compounds (0.01–0.18 mg ml^{-1} per component) were estimated using a unit response factor relative to palmitic acid.

EXPERIMENTAL

Propolis source. Eight propolis samples (20/1–20/8) were supplied as ethanolic tinctures by Comvita (NZ), Te Puke. The % solids and density of each of the tincture solns is given in parentheses. Each sample represented a bulked batch from apiaries in districts as follows: 20/1 (22.5%, 0.886 mg ml^{-1}), predominantly Waikato; 20/2 (19.9%, 0.875 mg ml^{-1}), predominantly Bay of Plenty; 20/3 (17.0%, 0.869 mg ml^{-1}), mixed Waikato/Coromandel/Auckland/Bay of Plenty; 20/4 (20.9%, 0.892 mg ml^{-1}), predominantly Taranaki; 20/5 (18.7%, 0.884 mg ml^{-1}), mixed Waikato/Taranaki/Bay of Plenty; 20/6 (20.5%, 0.879 mg ml^{-1}), mixed Waikato/Bay of Plenty/Coromandel; 20/7 (16.7%, 0.882 mg ml^{-1}), predominantly Auckland/Northland; 20/8 (19.3%, 0.876 mg ml^{-1}), mixed including Waikato/Bay of Plenty.

HPLC procedures. Sample prepn involved dilution of the supplied tincture by 200× and inclusion of a synthetic primary standard, 5,7-dimethyl-4′-methoxyflavanone such that its final concn was 0.04 mg ml^{-1}. Injection vol. was 10 μl. Samples were analysed on an end-capped LiChrospher 100 RP-18, 5 μm column (11.9 × 0.4 cm) using a Waters 600E solvent controller, Waters 996 photodiode array detector (set at 250–350 nm), Jasco 851-AS intelligent sampler and Millennium 2010 software. Elution was performed using both solvent systems A and B at 1.0 ml min^{-1} and 30°. Solvent system A comprised 5% formic acid (A′) and MeOH (B′) mixed using a linear gradient starting with 65% A′ decreasing to 55% A′ at 10 min, held for 10 min, then decreasing to 20% A′ at 55 min and 5% A′ at 60 min. Solvent system B comprised H_3PO_4 adjusted to pH 2.0 (A′) and MeCN (B′) mixed using a was used for quantification. Response factors for the quantifying standards, naringenin and chrysin, relative to the primary standard, were determined from HPLC runs in both solvent systems of a 1 : 1 : 1 (by wt) mixt. of all 3 compounds. Thus, dihydroflavonoids were calculated as naringenin equivalents using response factors of 2.082 (solvent system A) and 1.892 (B), and flavones and flavonols were calcd as chrysin equivalents using response factors of 0.478 (A) and 0.454 (B).

GC-MS procedures. Accurately weighed portions (*ca* 0.15 g) of the tincture solns were introduced into glass vials and diluted with EtOH–CH_2Cl_2 (1 : 1) (1 ml). An int. standard soln (300 μl of a 0.705 mg ml^{-1} soln of *n*-octadecane in CH_2Cl_2) and an ethereal soln of CH_2N_2 (1 ml) were added to each of the tincture solns. Samples were refrigerated for 2 hr to allow complete methylation to occur. Without the addition of CH_2Cl_2 significant pptn occurred during the derivatization step. Sub-samples of the methylated tincture solns were then analysed by GC-MS using a 25 m × 0.25 mm HP-1 (Hewlett Packard) methyl silicone capillary column installed in a HP5980 GC instrument interfaced to a HP5970B mass selective detector operated in scanning mode (m/z 40–400). GC-MS analysis were temp. programmed from 50° (0.3 min hold) to 285° (15 min hold) at 6° min^{-1}. Samples were injected using a HP7361 autoinjector utilizing the Grob split/splitless injection technique (2 μl injection vol., 0.1 min load time). Integration was performed using HP-Chemstation Software. Aromatic compounds and fatty acids were quantified relative to cinnamic acid and palmitic acid, respectively. Relative to *n*-octadecane the response factors for cinnamic acid and palmitic acid were 0.505 and 0.943, respectively (mean of 3 determinations).

MS data (m/z) *for methylated compounds identified by GC-MS analyses.* Compounds from which the stated Me esters are derived are given in parentheses. *Flavonoids:* pinocembrin 7-methyl ether (pinocembrin): 270 (100, M$^+$), 269 (65), 193 (87), 166 (57), 138 (40), 95 (36); galangin 7-methyl ether (galangin): 298 (100, M$^+$), 268 (19), 191 (28), 164 (43), 91 (66); pinobanksin 3-acetate 7-methyl ether (pinobanksin 3-acetate): 328 (36, M$^+$), 268 (73), 167 (100), 166 (46), 120 (51), 91 (35), 43 (54); chrysin 7-methyl ether: 268 (100, M$^+$), 267 (26), 239 (55), 225 (17), 91 (20); pinobanksin: 286 (64, M$^+$), 256 (59), 167 (100), 95 (54), 44 (73), 43 (60); pinobanksin 3-acetate 5,7-dimethyl ether (pinobanksin 3-acetate): 342 (9, M$^+$), 282 (18), 181 (66), 180 (100), 152 (26), 120 (24), 43 (29); pinocembrin 5,7-dimethyl ether (pinocembrin): 284 (64, M$^+$), 180 (100), 167 (61), 91 (47), 44 (42), 42 (45). *Aromatic compounds:* styrene: 104 (100, M$^+$), 103 (51), 78 (56), 77 (25), 51 (35); benzyl alcohol: 108 (94, M$^+$), 107 (65), 79 (100), 77 (97), 51 (46), 44 (33); methyl benzoate (benzoic acid): 136 (30, M$^+$), 105 (100), 77 (66), 51 (31), 50 (15); 2-phenylethanol:

77 (19); ethyl 3-phenylpropanoate: 178 (30, M^+), 107 (48), 105 (44), 104 (100), 91 (63); methyl cinnamate (cinnamic acid): 162 (45, M^+), 161 (22), 131 (100), 103 (74), 77 (49), 51 (35); 3,4-dimethoxybenzaldehyde (vanillin): 166 (100, M^+), 165 (66), 95 (44), 79 (27), 77 (45), 51 (30); ethyl cinnamate: 176 (30, M^+), 166 (20), 131 (100), 103 (61), 77 (37), 51 (26); methyl 5-phenyl-*trans*-3-pentenoate (5-phenyl-*trans*-3-pentenoic acid): 190 (29, M^+), 131 (38), 130 (100), 129 (38), 117 (52), 115 (38), 91 (63); ethyl-5-phenyl-*trans*-3-pentenoate: 204 (21, M^+), 131 (52), 130 (100), 129 (56), 117 (32), 115 (40); methyl 3,4-dimethoxybenzoate (vanillic acid): 196 (100, M^+), 165 (94), 79 (28), 77 (24), 59 (14), 51 (28); methyl 4-methoxycinnamate (*p*-coumaric acid): 192 (72, M^+), 162 (17), 161 (100), 133 (32), 59 (20); methyl 5-phenyl-*trans-trans*-2,4-pentadienoate (5-phenyl-*trans-trans*-2,4-pentadienoic acid): 188 (M^+), 157 (17), 129 (100), 128 (73), 127 (22), 59 (27); methyl ferulate (ferulic acid); 208 (100, M^+), 177 (84), 133 (35), 105 (39), 104 (48), 77 (39); ethyl 3,4-dimethoxycinnamate (ethyl ferulate): 222 (100, M^+), 207 (19), 191 (59), 147 (20); 3-methylbut-2-enyl 3,4-dimethoxycinnamate (3-methylbut-2-enyl ferulate = 1,1-dimethylallyl ferulate): 276 (41, M^+), 208 (100), 193 (13), 191 (68), 41 (15); 3-methylbut-3-enyl 3,4-dimethoxycinnamate (3-methylbut-3-enyl ferulate): 276 (49, M^+), 208 (100), 193 (31), 191 (31), 69 (25), 41 (56). *Fatty acid esters:* methyl myristate (myristic acid): 87 (61), 74 (100), 55 (35), 43 (37), 41 (46): ethyl myristate: 101 (62), 88 (100), 71 (29), 70 (28), 60 (34), 43 (28); methyl palmitate (palmitic acid): 270 (8, M^+), 227 (11), 143 (16), 87 (62), 75 (21), 74 (100), 69 (12) 57 (18), 55 (26), 43 (35), 41 (28); ethyl palmitate: 284 (10, M^+), 101 (51), 89 (17), 88 (100), 73 (19), 70 (19), 69 (15), 57 (21), 55 (25), 43 (39), 41 (35); methyl linoleate (linoleic acid): 294 (12, M^+), 95 (43), 82 (46), 81 (86), 79 (37), 69 (40), 68 (43), 67 (100), 55 (67), 54 (43), 41 (80); methyl oleate (oleic acid): 296 (5, M^+), 97 (44), 96 (37), 87 (35), 84 (36), 83 (48), 74 (55), 69 (65), 55 (100), 43 (62), 41 (74); methyl stearate (stearic acid): 298 (27, M^+), 143 (22), 87 (73), 75 (22), 74 (100), 69 (22), 57 (18), 55 (31), 43 (53), 41 (35); ethyl linoleate: 308 (10, M^+), 95 (52), 82 (42), 81 (82), 79 (37), 69 (41), 68 (47), 67 (100), 55 (77), 54 (41), 41 (80); ethyl oleate: 310 (5, M^+), 97 (38), 96 (36), 88 (47), 84 (38), 83 (45), 69 (58), 67 (34), 55 (100), 43 (55), 41 (75); ethyl stearate: 312 (22, M^+), 157 (23), 101 (65), 89 (21), 88 (100), 70 (18), 57 (25), 55 (27), 43 (55), 41 (44).

*Synthesis of methyl 5-phenyl-*trans*-3-pentenoate.* A mixt. of methyl acrylate (1.7 g), phenylacetaldehyde (2.4 g) (freshly distilled) and triphenylphosphine (5.2 g) in *t*-BuOH (15 ml) was heated in a 100-ml autoclave at 125° for 12 hr. Unreacted triphenylphosphine was removed by crystallization on cooling, and solvent and unreacted methyl acrylate were removed *in vacuo*. CC on silica gel with petrol:Et_2O (100:3) (33), 115 (51), 91 (79), 77 (18), 65 (20), 59 (15), 51 (22); ^1H NMR (300 MHz, $CDCl_3$): δ 3.23 (*d*, $J =$ 6.3 Hz, 2H, 2-CH_2), 3.43 (*d*, $J = 6.7$ Hz, 2H, 5-CH_2), 3.70 (*s*, 3H, OCH_3), 5.77 (*m*, 2H, 3-H, 4-H), 7.19–7.33 (*m*, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ 32.9 (C-5), 33.7 (C-2), 52.0 (OCH_3), 121.9 (C-3), 126.2 (C-4′), 128.5 (C-3′, C-5′), 128.6 (C-2′, C-6′), 131.8 (C-4), 140.1 (C-1′), 172.5 (CO).

*Synthesis of 5-phenyl-*trans-trans*-2,4-pentadienoic acid.* Based on the method of ref. [21]. A mixt. of cinnamic aldehyde (6.6 g) and KOAc (3 g) in Ac_2O (7.5 g) was heated at 170–180° for 3 hr. Workup gave 5-phenyl-*trans-trans*-2,4-pentadienoic acid (7.4 g), mp 165°. MS: m/z (%) 174 (M^+, 26), 157 (2), 130 (11), 129 (100), 128 (64), 115 (6), 102 (8), 91 (4), 77 (11), 64 (7), 63 (7), 51 (13): ^1H NMR (300 MHz, $CDCl_3$): δ 6.00 (*d*, $J =$ 15.3 Hz, 2-H), 6.93 (*dd*, $J =$ 15.3 Hz, 4-H, 5-H), 7.36 (*m*, 2′-H, 4′-H, 6′-H), 7.48 (*m*, 3′-H, 5′-H), 7.56 (*m*, 3-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 120.3 (C-2), 126.0 (C-4), 127.4 (C-3′, C-5′), 128.9 (C-2′, C-6′), 129.4 (C-4′), 135.9 (C-1′), 141.7 (C-5), 147.0 (C-3), 172.5 (C-1). The Me ester was prepd for GC-MS with CH_2N_2.

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