Free Primary Alcohols in Oils and Waxes from Germs, Kernels and Other Components of Nuts, Seeds, Fruits and Cereals

Kazuko Kawanishi[a,*], Kumiko Aoki[a], Yohei Hashimoto[a] and Akira Matsunobu[b,1]
[a]Kobe Women's College of Pharmacy, Kobe, 658, Japan and [b]Toyo Nut Co. Ltd., Kobe, 658, Japan

The composition of free primary alcohols in oils and waxes obtained from the germ, kernel, seed coat, shell and skin (peel) of various nuts, seeds, fruits and cereals and from the chrysalis of silkworm was examined. These alcohols are usually present in small amounts, along with large quantities of hydrocarbons, esters and glycerides in oils and waxes. Thus, it is necessary to remove hydrocarbons, esters and glycerides to analyze the alcohols. We found that preparative reverse-phase thin-layer chromatography (TLC) was the best way to isolate alcohols from oils and waxes. Gas liquid chromatography (GLC) then detected hexacosanol, octacosanol and triacontanol in the oils and waxes. Octacosanol usually was the predominant alcohol. Relationships between the organs from nuts, seeds, fruits and cereals and the contents of octacosanol are suggested. For example, degermed kernels contained two times more octacosanol than the germ, and the skin coat and shell contained one-half and one-fortieth the octacosanol of the germ, respectively.

KEY WORDS: Cereals, fruits, hexacosanol, nuts, octacosanol, oils, seeds, triacontanol, waxes.

Free primary alcohols are found in many plant waxes, *e.g.* in leaf, bark and stem waxes of *Holoptclea integrifolia* (known as Chilbil in India) (1), *Acacia modesta* (2), *Cassia javanica* (3), rye grass (4), and wheat (5,6). They are also found in germ of wheat (7), and octacosanol was detected mainly in potato slices incubated for 4 days rather than in fresh tissue (8). Hexacosanol and octacosanol have been found to be feeding stimulants for silkworm larvae (9) and are constituents of animal waxes such as aphid waxes (10). Therefore, some fatty alcohols also may be physiologically important substances.

Primary alcohols analyzed by gas liquid chromatography (GLC) without prior separation from other components such as hydrocarbons, esters and glycerides are sometimes hardly distinguished from other compounds that have similar retention times (11).

Because the content of alcohols in oils and waxes is small in comparison to the content of hydrocarbons, esters and glycerides, it is difficult to separate the former from the latter groups by thin-layer chromatography (TLC) even though the Rf values do differ (11). In this paper we examine methods of separation of primary alcohols, then discuss the composition of these alcohols as determined by GLC in the germ, degermed kernel, seed coat, shell, skin (peel) of various nuts, seeds, fruits and cereals and in the chrysalis of silkworm.

EXPERIMENTAL PROCEDURES

The plant materials, waxes and oils employed in this report, as listed in Table 1, 2 and 3 were obtained from commercial sources.

Hexacosanol, octacosanol and triacontanol were purchased from Nakarai Chemicals Ind. Ltd., Kyoto, Japan. Trimethylsilylimidazole (TMSI) for preparation of trimethylsilyl ethers and 1,3,5-triphenylbenzene (TPB) as internal standard were obtained from Wako Pure Chemical Ind. Ltd., Osaka, Japan, and Nakarai Chemicals Ind. Ltd., respectively. HPTLC-Fertigplatten RP-18F 254 (Merck, Darmstadt, Germany) preparative TLC plates were used and Japanese Industrial Standards Guaranteed Reagents were used for all other reagents.

Extraction of plant materials. Each plant organ (3–10 g) was removed from plant material by hand and extracted with petroleum ether (30–100 mL) in a Soxhlet apparatus for 48 hr, and the extract was then concentrated *in vacuo* to obtain the oils and waxes. Chrysalis wax of silkworm was also obtained by extracting the chrysalis (5 g) with petroleum ether in a Soxhlet apparatus.

Separation of alcohols. After many trials, such as organic solvent extractions and TLC on silica gel and alumina, TLC on reverse-phase plates developed with chloroform:methanol 1:2 was found to be the best way to separate alcohols from hydrocarbons, esters and glycerides in oils and waxes. The oils and waxes (5 mg) were applied to the TLC plate in narrow 2 mm × 80 mm bands. Authentic octacosanol solution also was spotted on both sides of the band as a marker. After developing the plate with chloroform:methanol 1:2, the Rf value of the alcohol band was located by spraying the authentic octacosanol band with 10% H_2SO_4 and heating the plate until a purple color appeared. Then, the area of the plate containing the alcohol band was scraped off, extracted with chloroform and filtered, and the solvent was removed *in vacuo*. For oil samples obtained by pressing the nuts under 6,000–20,000 psi or by extraction with hexane, 20-mg samples were subjected to preparative TLC on four plates by following the method described above. Alcohols from the samples were identified by GLC retention times against authentic alcohols (as trimethylsilyl ethers).

Analysis method by GLC. Alcohols, obtained by preparative TLC, were converted to trimethylsilyl ethers with TMSI by heating at 90°C for 1 hr, and the product was then dissolved in 40 μL of isopropyl ether containing 0.05% TPB as internal standard before being subjected to GLC analysis. GLC was performed with a Hewlett-Packard 5790A (Palo Alto, CA) and a glass capillary OV-101 column (5% Chromosorb Wax PMC 60/80, 0.25 mm × 46 m). The gas flow rates for N_2, H_2 and air were 40, 30 and 300 mL/min, respectively. The operating temperatures were as follows: injector, 230°C; detector, 330°C; initial oven temperature, 200°C, with a ramp rate of 2.5°C/min to 330°C. Alcohol peak areas were measured with a Hitachi Data Processor 833A (Tokyo, Japan) and are shown as percentages unless otherwise indicated.

Standard curve for octacosanol. 1,2,3,5 and 10-μg samples of authentic octacosanol were derivatized as trimethylsilyl ethers. They were dissolved in 40 μL of isopropyl ether containing 0.05% TPB as internal standard before being subjected to GLC analysis. The ratio

*To whom correspondence should be addressed at Kobe Women's College of Pharmacy, Higashinada-ku, Kobe, 658, Japan.

[1]Present address: Toyo Nut Co. Ltd., Higashinada-ku, Kobe, 658, Japan.

TABLE 1

Composition of Alcohols Extracted from Nuts, Seeds and Cereals

Material	Part[a]	Relative percent			Source
		Hexacosanol	Octacosanol	Triacontanol	
Hazel	G	39.2	60.8		Fiskobirlil, Turkey
	K	17.1	63.6	19.3	
	SC	29.7	70.3		
	S	28.6	51.0	20.4	
Cashew	G	25.0	35.0	40.0	Vitaya Lakehmi, India
Peanut	G	18.5	50.6	30.9	Liangyougongsi, China
	K	29.5	70.5		
	SC	25.3	52.5	22.2	
	S	10.7	23.9	65.4	
Almond	G	47.3	52.7		Sun Giant Inc., U.S.A.
	K	21.5	65.1	13.4	
	SC	28.6	54.4	16.8	
Pine seed	K	25.6	74.4		Liangyougongsi, China
	S	10.7	76.1	13.2	
Sunflower seed	G	24.5	57.9	17.6	Siegeo Sun Products Ltd., U.S.A.
	K	25.9	74.1		
	SC	29.3	60.2	15.5	
Maize	G	17.8	82.2		Industrial Sechi SA, Peru
	K	33.2	55.4	11.4	
Wheat	G	17.6	20.5	61.9	local market, Japan
Rice	G	13.5	35.4	51.1	local market, Japan
Prune	flesh	25.3	65.9	8.8	Sunsweet Growers Inc., U.S.A.
	seed	42.3	57.9		
Raisin	flesh	16.2	41.9	41.9	Sun Maid Growers Inc., U.S.A.
Hazel	oil	46.4	53.6		
Peanut	oil	35.3	50.3	14.4	
Almond	oil	56.3	43.7		
Pine seed	oil		29.4	70.1	

[a]G, germ; K, degermed kernel; SC, seed coat; S, shell; oil, obtained by press of whole nut.

TABLE 2

Octacosanol Contents of Fruits

Material	Part	Extract[a] (mg/g)	Octacosanol content (μg/g extract)	Octacosanol content (ppm)	Source
Plum	flesh	3	1415	7.1	Sunsweet Growers Inc., U.S.A.
Prunus domestica	seed	77	233	20.1	
Raisin	flesh	1	708	0.9	Sun Maid Growers Inc., U.S.A.
Vitis labrusca					
Apple	peel	58	663	221.8	local market, Japan
Malus pumila var. dulcissima					

[a]Petroleum ether-extractable material.

of the octacosanol peak area to internal standard peak area was measured with a Hitachi Data Processor 833A. The quantitative relationship of octacosanol peak area ratios to concentration is $y = 0.0214x - 0.0040$ ($r = 0.9995$).

Recovery of octacosanol from preparative TLC. Preparative TLC was performed by applying octacosanol solution (10 μg/25 μL) followed by the method described above in *Separation of alcohols.* Recovery of standard octacosanol applied to preparative TLC plates was 92.12% (SD = 3.80, five replicates).

Then the contents of octacosanol in alcohols from sample materials were obtained from the standard curve of the authentic octacosanol and from the recovery of octacosanol from preparative TLC.

TABLE 3

Octacosanol Contents of Waxes

Wax[a]	Extract[b] (mg/g)	Octacosanol content (μg/g extract)	Octacosanol content (ppm)
Rice wax	1000	29.1	29.1
Candelilla	1000	32.2	32.2
Lanolin	1000	17.5	17.5
Cera carnauba	880	36.1	32.1
Cera alba	1000	16.4	16.4
Cera flava	1000	21.9	21.9
Soluble substances of chrysalis of silkworm in petroleum ether	152	11.0	1.7

[a]Waxes, Noda Wax Co. Ltd., Japan (except rice wax); chrysalis of silkworm *(Bambyx mori),* Hyogo Prefectural Sericultural Technological Center, Hyogo, Japan.

[b]Petroleum ether-extractable material.

TABLE 4

Octacosanol Contents of Nuts, Seeds and Cereals

Material	Part[a]	Extract (mg/g)	Octacosanol content (μg/g extract)	Octacosanol content (ppm)
Hazel	G	659	5.6	3.7
Corylus avellana	K	630	10.7	6.7
	SC	153	11.6	1.8
	S	3	30.6	0.09
	W			P 6.0
				E 5.6
Cashew	G	291	4.8	1.1
Anacardium occidentale				
Peanut	G	421	9.2	3.9
Arachis hypogaea	K	471	14.5	6.8
	SC	91	23.4	2.1
	S	1	13.1	0.01
	W			P 5.6
				E 5.2
Almond	G	490	7.8	3.8
Prunus dulcis	K	526	12.4	6.5
	SC	118	15.6	1.8
	W			P 6.2
				E 5.6
Pine seed	K	717	5.3	3.8
Pinus koriensis	W			P 4.5
				E 4.7
Sunflower seed	G	516	8.0	4.1
Helianthus annuus	K	539	22.7	12.2
	SC	123	30.0	3.7
Maize	G	460	17.8	8.2
Zea mays var. indentata	K	5	11.5	0.06
Wheat	G	153	14.1	2.2
Triticum aestivum				
Rice	G	218	20.9	4.6
Oryza sativa				
Coconut	K			P 12.6
Cocos nucifera				E 7.9

[a]G, germ; K, degermed kernel; SC, seed coat; S, shell; W, whole; P, press; E, extraction.

Confirmation of alcohols obtained from preparative TLC. The soluble fraction of cera carnauba in petroleum ether was subjected to preparative TLC to obtain alcohols, which were benzylated with benzoic acid anhydride in pyridine under refluxing for 2 hr. The reaction mixture was put into ice water, extracted with ether, dried over sodium sulfate, filtered and concentrated. The residue was subjected to preparative TLC ($CHCl_3$:hexane 1:1, Kieselgel 60 F254, 0.5 mm, Merck) to purify the benzoate. The alcohol benzoate band was located under ultraviolet (UV) radiation (254 nm), scraped off, extracted with $CHCl_3$ and removed from the solvent, and the isolated benzoates were subjected to high-resolution mass spectrometric analysis (Hitachi 80). Thus, alcohols obtained from cera carnauba by preparative TLC were confirmed (as benzoates) as octacosanol, triacontanol, docontanol and tetracontanol (m/z = 514, 542, 570 and 598, respectively).

RESULTS AND DISCUSSION

Primary alcohols in germ, degermed kernel, seed coat, and shell obtained by extraction and preparative TLC consisted of hexacosanol, octacosanol and triacontanol with octacosanol being the predominant alcohol (Table 1). Octacosanol contents were analyzed in more detail as follows. The octacosanol content of extracts of selected plant materials is shown in Table 4 (ppm, dry weight basis). The content of octacosanol in germ from nuts and seeds is about 4 ppm, although cashew (1.5 ppm) and wheat (2.6 ppm) are lower, and maize (8.8 ppm) and rice (5.2 ppm) are higher. Octacosanol in degermed kernels was 1.6 to 2.9 times higher than in the germ, except for maize. The content of octacosanol per 1 gram of extract (waxes) was greater in seed coat and shell than in germ and degermed kernel. Of course, the weights of petroleum ether extracts obtained from the former were much less than from the latter (Table 2).

The peel of apple contained 222 ppm of octacosanol, while the flesh of plums and raisins contained 7 and 0.9 ppm, respectively (Table 3). In this connection, 20.1 ppm of octacosanol was found in the seed of plums.

The quantities of octacosanol in nut oils obtained by pressing whole nuts (4.5–6.2 ppm) were almost the same as in oils obtained by extraction (4.7–5.6 ppm) (Table 2). Coconut oil obtained by pressing the kernel had 12.6 ppm octacosanol, but by extraction only 7.9 ppm.

The content of octacosanol in waxes (Table 4) was usually higher than in nut oils (Table 2). Both candelilla wax and cera carnauba contained about 32 ppm of octacosanol, but the extract of silkworm chrysalis contained only 1.7 ppm.

The methodology introduced here, separation of small amounts of alcohols from a large amount of hydrocarbons, esters and glycerides, permitted us to accurately quantitate long-chain alcohols in nuts, seeds, fruits, cereals, oils and waxes. These alcohols were hexacosanol, octacosanol and triacontanol.

ACKNOWLEDGMENTS

We thank Director Minoru Uehara, Hyogo Prefectural Sericultural Technological Center, Hyogo, Japan, for furnishing us with the chrysalis of silkworms and also Dr. Kayoko Saiki, Kobe Women's College of Pharmacy, for spectra.

REFERENCES

1. Misra, G., S.C. Bhatnagar and S.K. Nigam, *Planta Medica 26*:394 (1974).
2. Joshi, K.C., M.K. Tholia and T. Sharma, *Ibid. 27*:280 (1975).
3. Joshi, K.C., M.K. Tholia and T. Sharma, *Ibid. 28*:190 (1975).
4. Hamilton, R.J., and D.M. Power, *Phytochemistry 8*:1771 (1969).
5. Tolloch, A.P., and R.O. Weenink, *Can. J. Chem. 47*:3119 (1969).
6. Tolloch, A.P., *Phytochemistry 12*:2225 (1973).
7. Levin, E., V.K. Collins, D.S. Varner, J.D. Mosser and G. Wolf, U.S. Patent 3031376 (1962).
8. Krulick, S., and S.F. Oaman, *J. Agric. Food Chem. 27*:212 (1979).
9. Mori, M., *J. Insect Physiol. 28*:969 (1982).
10. Nes, W.D., B.S. Campbell, A.E. Stafford, W.F. Haddon and M. Benson, *Biochem. Biophys. Res. Commun. 108*:1258 (1982).
11. Tolloch, A.P., in *Chemistry and Biochemistry of Natural Waxes*, edited by P.E. Kolattukudy, Elsevier, Amsterdam, Netherlands, 1976, p. 235.

[Received April 24, 1991; accepted August 26, 1991]