# **Essential Oil Compounds of the** *Annona muricata* **Fresh Fruit Pulp from Cameroon**

Leopold Jirovetz,<sup>†</sup> Gerhard Buchbauer,<sup>\*,†</sup> and Martin B. Ngassoum<sup>‡</sup>

Institute of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria, and Department of Applied Chemistry, University of Ngaoundere, B.P. 455, Ngaoundere, Cameroon

The essential oil of the exotic fresh fruit (pulp) *Annona muricata* (Annonaceae) from Cameroon was investigated by gas chromatographic/spectroscopic (GC/FID and GC/MS) and olfactoric methods to identify those constituents responsible for the intense and characteristic odor of this local foodstuff. Esters of aliphatic acids are especially dominating (total amount ~51%), with 2-hexenoic acid methyl ester (23.9%), 2-hexenoic acid ethyl ester (8.6%), 2-octenoic acid methyl ester (5.4%), and 2-butenoic acid methyl ester (2.4%) as main compounds. Additional mono- and sesquiterpenes such as  $\beta$ -caryophyllene (12.7%), 1,8-cineole (9.9%), linalool (7.8%),  $\alpha$ -terpineol (2.8%), linalyl propionate (2.2%), and calarene (2.2%) are highly concentrated in the essential oil of the fresh fruit of *A. muricata*. The use of this essential oil exhibiting a very pleasant odor in flavoring is discussed.

**Keywords:** Essential oil; Annona muricata; Annonaceae; fresh fruit pulp; aroma compounds; odor; olfactoric characterization; flavoring

## INTRODUCTION

In various locations (e.g., Malaysia and Sri Lanka) three species of Annona are grown for their edible fruits (MacLeod and Pieris, 1981; Wong and Khoo, 1993). The aroma compounds of the so-called soursop (Annona *muricata* L.), the sweetsop or sugar apple (Annona) squamosa L.), and the common custard apple, usually also known as the bullock's heart (Annona reticulata L.), had been investigated by some groups (Franco and Rodriguez-Amaya, 1983; Iwaoka et al., 1993; Pelissier et al., 1994). The pulp is consumed as fresh fruit or juice. Therefore, only extracts of the A. muricata fruit pulp were analyzed because of their pleasant aroma, but only one paper mentioned the volatiles from the essential oil of the fresh fruit pulp (Pelissier et al., 1994) with origin of the Ivory Coast. No information was given on odor components of the essential oil of fruits from Cameroon until now.

The aim of this paper therefore is to present the results of analyses of the aroma compounds from the essential oil of fresh *A. muricata* fruit pulp with Cameroonian origin and to discuss the odor activity of the identified volatiles for food (flavoring) and perfumery applications.

### EXPERIMENTAL PROCEDURES

**Plant Material.** The fresh fruits of *A. muricata* were collected by Dr. M. B. Ngassoum in August 1996 in the area of Douala (coastal region). The botanical identity was certified by Dr. J. M. Onana, Institute of Agronomic Research, and a voucher specimen deposited at the National Herbarium of Yaounde, Cameroon (No. 14066/SRFCAM).

**Isolation of Volatile Compounds.** The pulp of fresh fruits of *A. muricata* was separated from the peel and 500 g of the pulp placed in a steam distillation apparatus. After 6 h of steam distillation, 38 mg ( $\sim$ 0.08%) of essential oil was obtained.

**Olfactoric Characterization.** The essential oil of *A. muricata* was diluted with 1 mL of dichloromethane, and 10  $\mu$ L of this solution was placed on a commercial odor strip (Dragoco Co., Germany) for olfactoric characterization. The determination of the odor was performed by two professional perfumers and three aroma chemists.

**Gas Chromatography/Flame Ionization Detection.** The diluted samples (injected volume of 0.1  $\mu$ L) were analyzed with a GC-14A equipped with an FID and an integrator C-R6A-Chromatopac (Shimadzu Co., Japan) and by a GC-3700 equipped with an FID (Varian Co., Germany) and an integrator C-R1B-Chromatopac (Shimadzu Co.): carrier gas, hydrogen; injector temperature, 250 °C; detector temperature, 320 °C; temperature program, 40 °C/5 min to 280 °C/5 min with a heating rate of 6 °C/min; columns, 30 m × 0.32 mm bonded FSOT-RSL-200 fused silica (film thickness = 0.25  $\mu$ m; Bio-Rad Co., The Netherlands) and 30 m × 0.32 mm bonded Stabilwax (film thickness = 0.50  $\mu$ m; Restek Co., USA). Identifications of compounds were performed by co-injection of pure substances and correlation with published data (Davies, 1990; Jennings and Shibamoto, 1980).

**Gas Chromatography/Mass Spectrometry.** After GC/ FID analyses, the sample were analyzed with (1) a GC-17A coupled with a QP5000 (Shimadzu Co.) with the data system Compaq-ProLinea (class5k software); (2) a GC-HP5890 coupled with an HP5979-MSD (Hewlett-Packard Co., Avondale, PA) and a Pentium-PC (Böhm Co., Austria; ChemStation software) as well as (3) a GCQ (Finnigan-Spectronex Co., USA-Switzerland) with the data system Gateway-2000-PS75 (Siemens-Nixdorf Co., Germany; GCQ software); carrier gas, helium; injector temperature, 250 °C; interface heating, 300 °C; ion source heating, 200 °C; EI mode; scan range, 41–450 amu; other parameters, see the above paragraph on GC/FID.

The mass spectra were correlated with Wiley, NBS, NIST, and private library spectra.

#### **RESULTS AND DISCUSSION**

More than 50 compounds were identified in the essential oil of the fresh fruit (pulp) of *A. muricata* (Annonaceae) from Cameroon. Esters of aliphatic acids are especially dominating (total amount  $\sim$ 51%): 2-hexenoic acid methyl ester (23.9%), 2-hexenoic acid ethyl

<sup>&</sup>lt;sup>†</sup> University of Vienna.

<sup>&</sup>lt;sup>‡</sup> University of Ngaoundere.

		concen-	
no.	compound <sup>a</sup>	tration <sup>b</sup>	IM <sup>c</sup>
1	β-bisabolene	0.4	GC, MS
2	butanoic acid	0.2	GC, MS
	butanoic acid methyl ester	1.1	GC, MS
4		2.4	MS
5	calarene	2.2	GC, MS
6	$\beta$ -caryophyllene	12.7	GC, MS
7	1,8-cineole	9.9	GC, MS
ð	<i>p</i> -cymene	0.3	GC, MS
	α-elemene	0.1 0.3	GC, MS GC, MS
	$\beta$ -elemene heptanoic acid methyl ester	0.3	MS MS
	hexanoic acid	0.2	GC, MS
	hexadecanoic acid ethyl ester	0.1	MS MS
	hexadecanoic acid methyl ester	0.2	MS
15	hexanoic acid ethyl ester	0.2 0.7	MS
16	hexanoic acid methy ester	1.7	MS
17	2-hexenoic acid ethyl ester	8.6	MS
18	2-hexenoic acid methyl ester	23.9	MS
19	3-hexenoic acid methyl ester	0.2	MS
20	(Z)-3-hexenol	1.1	GC, MS
21	(E)-2-hexenol	0.1	GC, MS
22	3-hydroxybutanoic acid methyl ester	0.2	MS
23	3-hydroxyheptanoic acid methyl ester	0.1	MS
24	2-hydroxyhexanoic acid methyl ester	0.8	MS
25	3-hydroxyhexanoic acid methyl ester	0.2	MS
26	limonene	1.2	GC, MS
27	linalool	7.8	GC, MS
28		0.1	GC, MS
29	trans-linalool oxide (furanoide)	0.2	GC, MS
	linalyl acetate	1.3	GC, MS
	linalyl propionate	2.2	GC, MS
32	nerol	0.3	GC, MS
	nerolidol	0.9	GC, MS
	nonanoic acid ethyl ester	0.2	MS
35 36	nonanoic acid methyl ester	0.1	MS
30 37	2-nonenoic acid ethyl ester	0.3 0.5	MS MS
38	2-nonenoic acid methyl ester 9,12,15-octadecatrienoic acid ethyl ester	0.3	MS
39	9,12,15-octadecatrienoic acid ethyl ester	0.2	MS
<b>40</b>	9-octadecenoic acid ethyl ester	0.3	MS
41	octanoic acid ethyl ester	0.8	GC, MS
42		0.3	GC, MS
43	2-octenoic acid ethyl ester	0.4	MS
44	2-octenoic acid methyl ester	5.4	MS
45	α-pinene	1.4	GC, MS
<b>46</b>	$\beta$ -pinene	1.1	GC, MS
47	α-terpinene	0.2	GC, MS
<b>48</b>		0.5	GC, MS
	terpinen-4-ol	0.4	GC, MS
	α-terpineol	2.8	GC, MS
51	1	0.6	GC, MS
52	tetradecanoic acid ethyl ester	0.7	MS
53	tetradecanoic acid methyl ester	0.1	MS
	unknown	$\sim 0.4$	

<sup>*a*</sup> In alphabetical order. <sup>*b*</sup> Concentration in % peak area as calculated by GC-FID analysis. <sup>*c*</sup> Identification method: GC compound identification by correlation with published retention times; MS compound identification by mass spectra correlations.

ester (8.6%), 2-octenoic acid methyl ester (5.4%), and 2-butenoic acid methyl ester (2.4%).

Additional terpenic compounds, such as  $\beta$ -caryophyllene (12.7%), 1,8-cineole (9.9%), linalool (7.8%),  $\alpha$ -terpineol (2.8%), linalyl propionate (2.2%), and calarene (2.2%), were found to be the main components (concentration > 2%; Table 1).

The olfactoric data can be correlated as follows: the exotic-fruity odor impression is determined by the esters of the aliphatic acids and by the monoterpenes linalool and  $\alpha$ -terpineol; the fatty notes are produced by nonane, tetradecane, and esters of the hexadecanoic acid, re-

spectively, the green-sour-herbal notes of especially hexane derivatives.

In comparison with published data on volatiles from A. muricata samples (Pelissier et al., 1994; Wong and Khoo, 1993), it seems noteworthy that aliphatic esters are the main constituents independent of the origin of the sample [total amount of aliphatic esters by Pelissier et al. (1994), ~69%; by Wong and Khoo (1993), ~57%; in this analysis,  $\sim$ 51%]. Significant is only the content of the odor compounds (E)-but-2-enoate [Wong and Khoo (1993), 19.7%; Pelissier et al. (1994), 4.8%; here, 2.4%], linalool [Wong and Khoo (1993), 9.3%; Pelissier et al. (1994), not detected; here, 7.8%], and (Z)-3-hexenol [Wong and Khoo (1993), 9.7%; Pelissier et al. (1994), 0.5%; here, 1.1%]. The composition differs drastically and has therefore an important influence on the characteristic odor of this A. muricata sample from Cameroon. Although the investigated fresh fruit pulp sample from the Ivory Coast (Pelissier et al., 1994) stems also from the same geographical region of tropical Africa (Ivory Coast and Cameroon are the same number of degrees north of the equator), and hence the same composition could be expected, some differences-as shown above-were detected. The presence of linalool  $(\sim 8\%)$  in the essential oil of the Cameroonian species and the lack of this monoterpene alcohol in the species of the Ivory Coast should nevertheless be emphasized.

The flavoring of food products with this essential oil may play an important role in the future, because of the high value of fruity aromas in human nutrition (soft drinks, fruit products, flavored teas, flavored milk products, chewing gums, cakes, sweeties, tarts, etc.). Also, in fine perfumery (class of fruity and green notes), this essential oil seems to be a valuable raw material for various applications.

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#### LITERATURE CITED

- Davies, N. W. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J. Chromatogr. 1990, 503, 1–24.
- Franco, M. R. B.; Rodriguez-Amaya, D. B. Trapping of Soursop Annona muricata Juice Volatiles on Porapak-Q by Suction. J. Sci. Food Agric. 1983, 34 (3), 293–299.
- Iwaoka, W. T.; Zhang, X.; Hamilton, R. A.; Chia, C. L.; Tang, C. S. Identifying Volatiles in Soursop and Comparing their Changing Profiles During Ripening. *HortScience* **1993**, *8*, 817–819.
- Jennings, W.; Shibamoto, T. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography, Academic Press: New York, 1980.
- MacLeod, A. J.; Pieris, N. M. Volatile Flavour Components of Soursop Annona muricata. J. Agric. Food Chem. 1981, 39 (3), 488–490.
- Pelissier, Y.; Marion, Ch.; Kone, D.; Lamaty, G.; Menut, Ch.; Bessiere, J.-M. Volatile Components of Annona muricata L. J. Essent. Oil Res. 1994, 6, 411–414.
- Wong, K. C.; Khoo, K. H. Volatile Components of Malaysian Annona Fruits. *Flavour Fragrance J.* **1993**, *8* (1), 5–10.

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