Pergamon 0031-9422(95)00455-6 Phytochemistry, Vol. 41, No. 2, pp. 433–438, 1996
Elsevier Science Ltd
Printed in Great Britain
0031-9422/96 $15.00 + 0.00

VOLATILE COMPONENTS OF RIPE FRUITS OF *MORINDA CITRIFOLIA* AND THEIR EFFECTS ON *DROSOPHILA*

JEAN-PIERRE FARINE,*† LUC LEGAL,‡§ BRIGITTE MORETEAU¶ and JEAN-LUC LE QUERE||

*INRA Laboratoire de Recherches sur les Arômes, 17 Rue Sully, F-21034, Dijon, France; ‡Laboratoire des Mécanismes de Communication, URA 1491, Bat. 446, Université Paris Sud, Orsay Cedex, F-91405, France; ¶Laboratoire Populations, Génétique et Evolution, UPR 9034, CNRS, Gif sur Yvette, F-91198, France; ||INRA., Laboratoire de Recherches sur les Arômes, 17 Rue Sully, F-21034, Dijon, France

(*Received in revised form* 1 *May* 1995)

Key Word Index—*Morinda citrifolia*; Rubiaceae; volatile components; alkanoic acids; *Drosophila melanogaster* subgroup; toxicity.

Abstract—The only larval resource of the specialist species, *Drosophila sechellia*, is ripe fruits of *Morinda citrifolia*. The chemical composition of this fruit, which is very toxic to most *Drosophila* species, was investigated and 51 compounds were abundant enough to be identified by GC–MS. The ripe fruit is characterized by a large amount of carboxylic acids, especially octanoic and hexanoic acids. The biological effects of the ripe fruit and its main acids were investigated with behavioural studies. Octanoic acid is responsible for the general toxicity of the fruit to most *Drosophila* species; *D. sechellia* is the only species which is resistant to this acid. Hexanoic acid has a unique effect, causing reversible coma but no mortality. Decanoic acid is inactive. A mixture of these three acids in proportions similar to those found in the fruit, mimics the effects of ripe fruits of *M. citrifolia*.

INTRODUCTION

Morinda citrifolia is a shrub which grows in sandy areas along many tropical coasts. Native from the Indian Ocean [1], this species is not present in continental Africa where it is replaced by a related species, *M. lucida*. The introduction of *M. citrifolia* into Australia [2], Hawaii [3, 4] and in the Caribbean seems to have be done by man.

In Asian countries, *M. citrifolia* has been extensively used in folk medicine and as a dye. Medical applications have been reported for diabetes [5], antiseptic and antibiotic properties [6, 7] and hypotensive and anticoagulant activities [8–10]. Specific compounds have been identified including asperulosid [11–14], ursolic acid [15], hexanoic and octanoic acids [10] and a large variety of anthraquinones from the fruits and roots [7, 16]. The main products for dyeing have been prepared from the roots. The colour obtained changes from orange to deep red and the compounds involved are nordmnacanthal, morindone, rubiadine and rubiadine 1-methyl ether [17, 18].

Morinda citrifolia is the natural host plant of *Drosophila sechellia* [19, 20], a member of the *D. melanogaster* subgroup which is subdivided into two complexes [21], the *D. melanogaster* complex (*D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. sechellia*) and the *Drosophila yakuba* complex (*D. yakuba*, *D. tessieri*, *D. orena* and *D. erecta*). Contact with ripe fruits of *Morinda* kills flies of any *Drosophila* species in a few minutes except *D. sechellia* [22, 23]. Moreover, flies of the sensitive *Drosophila* species are repelled by the ripe fruit while *D. sechellia* is attracted to it [23]; green and rotten fruits are not toxic [24]. The most toxic compound in the ripe fruit is octanoic acid [24], whereas hexanoic acid seems to play a role for attraction and egg-laying preference [25, 26].

The main purpose of the present study was to identify and quantify the main volatile compounds present in ripe fruits of *M. citrifolia* and to test the biological effects of some of the identified chemicals on several *Drosophila* species.

§Author to whom correspondence should be addressed.

†Present address: Université de Bourgogne, Laboratoire de Zoologie, CNRS, URA 674, 6, Bd, Gabriel F-21100, Dijon, France.

Table 1. Identified violatile compounds from ripe fruits of *Morinda citrifolia*

Compounds	M_r	Identification*	%	ppm
Acids				
Acetic	60	a, b, c	0.04	0.02
2-methyl propanoic	88	a, b	0.11	0.05
Butanoic	88	a, b, c	0.71	0.31
2-methylbutanoic	102	a, b	0.54	0.23
Hexanoic	116	a, b, c	19.24	8.26
3-methylthiopropanoic	120	a, b	0.41	0.18
Benzoic	122	a, b, c	0.19	0.08
Heptanoic	130	a, b, c	0.09	0.04
Octanoic	144	a, b, c	58	24.98
Hexanedioic	146	a, b	0.08	0.03
Nonanoic	158	a, b	0.03	0.01
Decanoic	172	a, b, c	1.54	0.66
Undecanoic	186	a, b, c	0.03	0.02
Lauric	200	a, b, c	0.16	0.07
Myristic	228	a, b, c	0.14	0.06
Palmitic	256	a, b, c	0.49	0.21
Linoleic	280	a, b, c	0.05	0.02
Elaidic	282	a, b, c	0.29	0.12
Oleic	282	a, b, c	0.06	0.03
(*Z*,*Z*,.*Z*)-8,11,14-eicosatrienoic	306	a, b	0.68	0.30
Total			82.88	35.68
Alcohols				
1-Butanol	72	a, b, c	0.07	0.03
3-Methyl-3-buten-1-ol	86	a, b	4.13	1.78
3-Methyl-2-buten-1-ol	86	a, b	0.30	0.13
1-Hexanol	102	a, b, c	0.11	0.05
Benzyl alcohol	108	a, b, c	0.05	0.02
Eugenol	164	a, b	0.03	0.01
(*Z*,*Z*)-2,5-Undecadien-1-ol	168	a, b	0.41	0.18
Total			5.1	2.20
Esters				
Methyl hexanoate	130	a, b, c	0.37	0.16
Methyl 3-methylthio-propanoate	134	a, b	0.03	0.01
Ethyl hexanoate	144	a, b, c	0.12	0.05
Methyl octanoate	158	a, b, c	0.85	0.37
Ethyl octanoate	172	a, b, c	0.45	0.19
Methyl decanoate	186	a, b, c	0.57	0.25
Ethyl decanoate	200	a, b, c	0.19	0.08
Methyl palmitate	270	a, b, c	0.04	0.02
Ethyl palmitate	284	a, b, c	0.03	0.02
Methyl elaidate	296	a, b, c	0.05	0.02
Methyl oleate	296	a, b, c	0.03	0.01
Total			2.76	1.18
Ketones				
3-Hydroxy-2-butanone	88	a, b, c	0.06	0.03
2-Heptanone	114	a, b, c	0.35	0.15
Total			0.41	0.18
Lactones				
(*E*)-6-Dodeceno-γ-lactone	196	a, b	0.09	0.04
(*Z*)-6-Dodeceno-γ-lactone	196	a, b	0.09	0.04
Total			0.18	0.8
Miscellaneous compounds				
Hexanamide	115	a, b	0.03	0.01
Limonene	136	a, b, c	0.39	0.17
(Ethylthiomethyl) benzene	152	a, b	0.07	0.03
Unknown 1	166	b	1.48	0.64
Unknown 2	168	b	0.56	0.25
Unknown 3	184	b	0.57	0.25
Scopoletin	192	a, b	1.97	0.85
Vomifoliol	206	a, b	0.95	0.40
Unknown 4	212	b	1.65	0.71
Total			7.67	3.31

*Chemical identifications were based on EI mass spectra (a); CI mass spectra (b); and/or comparisons of their R_ts with those of synthetic compounds (c).

RESULTS

Chemical analyses

A typical GC trace from an extract of *M. citrifolia* ripe fruit reveals the presence of *ca* 50 detectable volatile compounds (Table 1). There are 20 acids which represent 83% of the volatiles, six alcohols (5%) and 11 esters (3%). Ketones and lactones are present in smaller amounts (less than 0.5%). For aldehydes, only traces of hexanal were detected. Five identified compounds represent *ca* 85% of all volatile components, viz., octanoic acid (58%), hexanoic acid (19%), 3-methyl-3-buten-1-ol (4%), scopoletin (2%) and decanoic acid (2%).

Toxicity of ripe fruits

In previous work [23], it was shown that, in the bioassay used with 1.5 g of ripe fruit pulp, no *D. sechellia* flies were killed in 40 min. The results presented here show that flies of both sexes of *D. mauritiana* and *D. simulans* are significantly more sensitive than those of *D. melanogaster* (Canton S strain) (Table 2). For durations of 20 and 30 min, the mortality percentage of *D. simulans* flies is intermediate between those of *D. melanogaster* and *D. mauritiana*, with very significant difference for females.

Toxicity of octanoic acid

With octanoic acid, it was previously shown that the LD_{50} values, measured at 40 min, were markedly different between *D. melanogaster* (males: 36 ± 7 μg; females: 39 ± 18 μg) and *D. sechellia* (males: 185 ± 24 μg; females: 238 ± 24 μg) [24]. When the toxicity levels of 50 μg of octanoic acid were compared for the three sensitive fly species, the differences observed with the fruit pulp were no more significant, but some new differences appeared (Table 3). While with the pulp 27.2% of the flies (taking into account both sexes of the three species) had died in 10 min, using pure octanoic acid, the average mortality percentage was only 2.2%. The differences between pulp and pure compound decreased with time. These observations suggest a sigmoidicity at the beginning of the mortality curve.

Another difference is a sexual dimorphism observed with the pure acid whichever the species; this was not observed with the pulp. With octanoic acid, all males were significantly more sensitive than females from the beginning of the experiment up to 40 min.

Toxicity of hexanoic and decanoic acids

The toxicity of 50 μg of octanoic acid is comparable to that of 1.5 g of ripe fruit pulp. A comparable dose of either hexanoic or decanoic acid caused very little mortality whichever the species. In 40 min, only males showed some mortality with this quantity of hexanoic acid: *D. mauritiana*, 21% dead flies; *D. simulans*, 15%; *D. melanogaster*, 12% and *D. sechellia*, 1% (Fig. 1). This compound induced a reversible coma associated with characteristic symptoms of altered equilibrium, strong wing vibrations, followed by complete immobilization;

Table 2. Mortality kinetics in response to ripe *Morinda citrifolia* fruit pulp for *Drosophila melanogaster* (meCS), *D. simulans* (sS) and *D. mauritiana* (ma). No mortality was observed for *D. sechellia*

(A)*

	meCS		sS		ma	
Time (min)	Males	Females	Males	Females	Males	Females
10	20 ± 1.9	21 ± 1.9	30 ± 2.1	28 ± 1.3	34 ± 1.6	30 ± 1.6
20	51 ± 2.1	41 ± 1.9	62 ± 1.9	57 ± 2.1	72 ± 1.3	72 ± 2.1
30	64 ± 1.9	62 ± 1.3	95 ± 0.8	75 ± 1.6	96 ± 0.8	85 ± 1.1
40	84 ± 1.1	78 ± 1.3	97 ± 0.5	88 ± 1.3	97 ± 0.5	91 ± 1.3
50	92 ± 0.8	87 ± 1.6	100	95 ± 1.1	100	95 ± 0.8
60	100	96 ± 0.5	—	100	—	100
70	—	100	—	—	—	—

(B)†

	10 min			20 min			30 min			40 min		
	meCS	ma	sS	meCS	ma	sS	meCS	ma	sS	meCS	ma	sS
meCS	NS	***	**	**	***	***	NS	***	***	NS	***	***
ma	**	NS	NS	***	NS	***	***	***	NS	***	**	NS
sS	NS	NS	NS	***	***	NS	***	**	***	**	NS	NS

*(A) Mean percentages of mortality and standard errors: 1.5 g of ripe *Morinda*, 15 × 10 flies tested. † (B) Statistical analysis: two-sided Student's *t*-test for times 10–40 min (underlined: intraspecific comparisons between males and females, above diagonal in ordinary type: interspecific comparisons between males; under diagonal in bold: interspecific comparisons between females). NS: $P > 0.05$, *: $0.05 > P > 0.01$, **: $0.01 > P > 0.001$, ***: $P < 0.001$.

Table 3. Mortality kinetics in response to pure octanoic acid (50 μg) for *Drosophila melanogaster* (meCS), *D. simulans* (sS) and *D. mauritiana* (ma), (A) and statistical analysis (B)*

(A)

	meCS		sS		ma	
Time (min)	Males	Females	Males	Females	Males	Females
10	3 ± 0.5	2 ± 0.3	3 ± 0.3	0	3 ± 0.3	2 ± 0.5
20	52 ± 2.1	38 ± 1.3	52 ± 1.6	41 ± 1.9	52 ± 2.1	33 ± 1.3
30	72 ± 1.9	51 ± 1.6	72 ± 2.6	55 ± 2.1	76 ± 1.9	55 ± 1.9
40	89 ± 1.3	74 ± 1.6	88 ± 1.6	72 ± 1.6	98 ± 0.3	82 ± 1.9
50	98 ± 0.5	89 ± 1.1	98 ± 0.5	88 ± 0.8	100	95 ± 1.1
60	100	94 ± 0.8	98 ± 0.5	92 ± 1.3	—	97 ± 0.5
70	—	98 ± 0.3	98 ± 0.5	96 ± 0.5	—	99 ± 0.3

(B)

	10 min			20 min			30 min			40 min		
	meCS	ma	sS	meCS	ma	sS	meCS	ma	sS	meCS	ma	sS
meCS	NS	NS	NS	***	NS	NS	***	NS	NS	***	**	NS
ma	NS	NS	NS	NS	***	NS	NS	***	NS	**	***	**
sS	NS	NS	NS	NS	**	***	NS	NS	***	NS	***	***

*No mortality was observed for *D. sechellia* for this dose. See also Table 2.

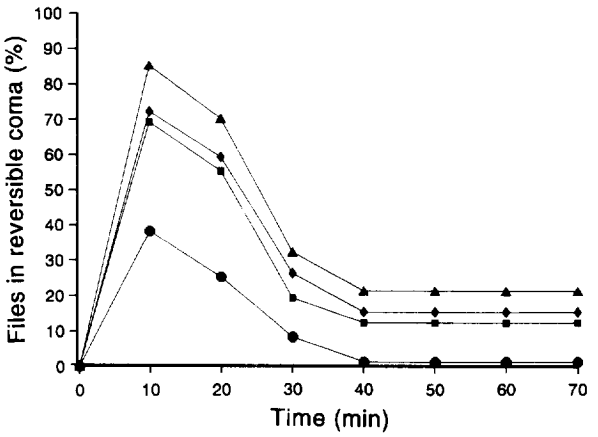

Fig. 1. Reversible coma kinetics observed in response to 50 μg of hexanoic acid with males of ■, *D. Melanogaster*; ◆, *D. simulans*; ▲, *D. mauritiana*; and ●, *D. sechellia*. 150 flies tested.

this happened within a few min for flies of every species tested, including *D. sechellia*. But after 20–30 min most of the flies recovered and then exhibited apparently normal behaviour (Fig. 1). Decanoic acid induced neither toxicity nor any apparent behavioural disorder, at least for a 50 μg dose.

Toxicity of an octanoic–hexanoic–decanoic mixture

A mixture of the three acids was prepared with proportions similar to those observed in the ripe fruit. In the presence of the mixture, the mortality kinetics were rather similar to those observed with the pulp. The sensitivity hierarchy of the various species towards the mixture is also similar to that observed with the pulp; *D. simulans* is intermediate between *D. melanogaster* (less sensitive) and *D. mauritiana* (the most sensitive) (Table 4, Fig. 2). As observed with octanoic acid, females were more resistant than males.

DISCUSSION

The sensitivity of adult flies of the *Drosophila melanogaster* subgroup to the pulp of *Morinda* ripe fruit is dependent on the species. *Drosophila mauritiana* is the most sensitive species, *D. melanogaster* the most resistant; *D. simulans* is intermediate between the two former species and *D. sechellia* is resistant to the pulp. In *D. melanogaster*, a more resistant strain than Canton S was previously described, the African strain 'Taï', [23].

Carboxylic acids represent 83% of all the volatiles of ripe fruits. Among these acids, except a few which were either branched, unsaturated or bearing a thiol group, all the others are linear alkanoic acids. Most of them have an even number of carbons. The major one, octanoic acid, represents 58% of all the volatiles. This compound has been previously found to play an important role in *Morinda* toxicity for *Drosophila* [24]. A few differences are described here between the effects of pure octanoic acid and the pulp of the ripe fruit: toxicity latency differences, sensitivity differences between species and sexes. The effect of hexanoic acid on flies is unique. At 50 μg, while octanoic acid kills flies, hexanoic acid causes little mortality but induces a reversible coma. At the same

Table 4. Mortality kinetics in response to mixture of acids (octanoic acid (50 μg), hexanoic acid (10 μg) and decanoic acid (2.5 μg)) for *Drosophila melanogaster* (meCS), *D. simulans* (sS) and *D. mauritiana* (ma) (A) and statistical analysis (B)*

(A)

	meCS		sS		ma	
Time (min)	Males	Females	Males	Females	Males	Females
10	20 ± 1.6	15 ± 0.5	27 ± 1.1	17 ± 0.8	65 ± 1.1	34 ± 1.1
20	70 ± 1.9	35 ± 0.8	72 ± 1.6	45 ± 1.1	100	92 ± 0.5
30	90 ± 0.8	62 ± 1.6	92 ± 0.8	80 ± 1.6	—	100
40	100	82 ± 0.5	100	92 ± 1.1	—	—
50	—	97 ± 0.5	—	100	—	—
60	—	97 ± 0.5	—	—	—	—
70	—	97 ± 0.5	—	—	—	—

(B)

	10 min			20 min			30 min			40 min		
	meCS	ma	sS	meCS	ma	sS	meCS	ma	sS	meCS	ma	sS
meCS	NS	***	NS	***	***	NS	***	**	NS	***	NS	NS
ma	***	***	***	***	***	***	***	NS	**	**	NS	NS
sS	*	***	**	**	***	**	***	***	***	***	**	**

*No mortality was observed for *D. sechellia* for this dose. See also Table 2.

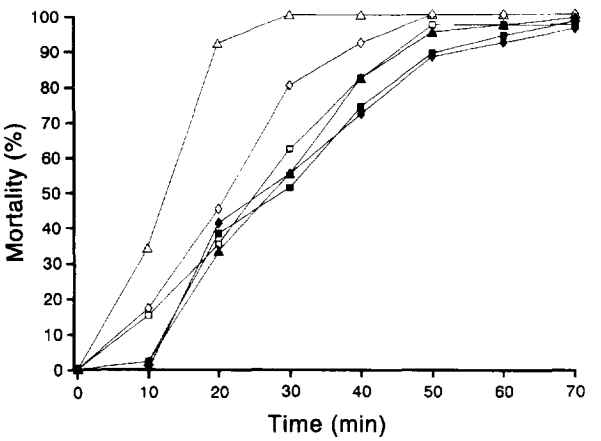

Fig. 2. Mortality kinetics of females of ■, *D. melanogaster*; ◆, *D. simulans*; and ▲, *D. mauritiana*; observed in response to either 50 μg of octanoic acid (black marks) or a mixture of 50 μg of octanoic acid, 10 μg of hexanoic acid and 2.5 μg of decanoic acid (grey marks). 150 flies tested.

dose, decanoic acid does not produce any observable effect on flies of any species. The effect of the three acids mixed in proportions comparable to that of the fruit pulp strongly resembles the effects observed with the pulp.

Besides toxic acids, esters are also present in ripe fruits of *Morinda*. Only methyl or ethyl esters of the most abundant acids described above have been identified, but the quantities of these products depend on the physiological stage of the fruit [24]. In rotten fruits, not toxic to *Drosophilas*, acids are partially transformed into esters (mainly ethyl) which decreases the overall toxicity of the fruits.

3-Methyl-3-buten-1-ol is the only alcohol present in significant amounts but its possible role on fly behaviour has not been investigated. There is no structural relation between the alcohols and the acids found in the fruit. This fact plus the apparent absence of aldehydic compounds might suggest that acids are directly produced in the fruit and are not derived from alcohols through oxidation.

Of the other compounds identified, an interesting one is scopoletin. As for other coumarins, the production of this phytoalexin has been associated with stresses, especially those linked to fungi [27–29]. Different environmental chemicals, acids or aldehydes, saturated or not, have been implicated in the induction of scopoletin production [30, 31]. The high quantity of scopoletin found in *Morinda* might be related to the large amount of alkanoic acids also present. Consequently, the fruit might possess several interdependent components of a defence mechanism against fungi infection and phytophagous attacks. *Morinda* is protected against attack by most phytophagous insects; *D. sechellia* is a rare species with sufficient tolerance to alkanoic acids which, thus, is able to use the ripe fruit as a food source.

EXPERIMENTAL

Flies. Strains of *D. melanogaster* Canton 'S' (meCS), *D. simulans* Seychelles (sS) and *D. mauritiana* (ma) were grown on a standard medium at 25 ± 0.5° under a 12:12 hr light–dark cycle. For *D. sechellia* (se), a small amount of axenic medium with alcohol was added to the standard medium to increase viability.

Toxicity bioassay. According to a previously described method [23], flies were tested in groups of 10 and each test was repeated at least 15 times. Flies were introduced into Petri dishes (vol. 8 ml) containing a known amount of either fruit pulp or synthetic product. Mortality was considered when fly wings were vertical. Values of LD_{50} (doses at which 50% of flies were dead after 40 min) were calculated and submitted to statistical analysis (Student's *t*-test, SPSS/PC$^+$ software). Tests were performed with 1.5 g of ripe fruit pulp or 50 μg of each tested acid or a mixture of 50 μg of octanoic, 10 μg of hexanoic acid and 2.5 μg of decanoic acid. In the CH_2Cl_2 extraction reported here (see below) the proportions of the three main acids were *ca* 74% for octanoic acid, 24% for hexanoic acid and 2% for decanoic acid. Using Me_2CO for extraction, their respective proportions were 80%, 15% and 4% [24].

Chemical analyses. Frozen ripe fruits (400 g) collected in Moorea (French Polynesia) were crushed in 500 ml of deionized H_2O and 500 g of $(NH_4)_2SO_4$. The slurry was extracted $\times 3$ during 15 min using 150 ml of dist. CH_2Cl_2. The pooled extracts were dried (Na_2SO_4), concd to 500 μl by the Kuderna–Danish method [32] and kept at $-20°$ until used. The expt was replicated $\times 3$. CH_2Cl_2 extracts were analysed by FID–GC and GC–MS. A CPWax 58 CB (30 m $\times$ 0.25 mm id, 0.22 μm film thickness) fused silica capillary column was used. Samples (1 μl) were injected via a split–splitless inj. system, operating with a split flow of 25 ml/min^{-1} and a septum purge of 3 ml min^{-1}. Split and purge ports were closed during injn and opened 30 sec after injn. The column was held isothermally at 40° for 2 min, heated to 60° in 1 min, and then to 240° at 2°C min^{-1}. Hydrogen was the carrier gas (50 cm sec^{-1} at room temp.). Inj. and detector temps were 250 and 270°, respectively.

For GC–MS, a quadrupole mass spectrometer was used. The GC column was a CP Wax 58 CB (60 m $\times$ 0.25 mm id, 0.22 μm film thickness) and operated using conditions similar to those described above. The column was connected directly to the ion source of the spectrometer through a heated transfer line maintained at 260°. EI–MS was performed at 70 eV with a source temp. of 150° (instrument scanning from 25 to 300 mu in 0.8 sec). CI–MS was performed at 90 eV with a source temp. of 90° and NH_3 (source pres. 0.3 Torr, instrument scanning from 60 to 300 mu in 0.7 sec). Compounds were identified by comparing their MS to those in the library of the Laboratoire de Recherches sur les Arômes (INRA, Dijon, France). Whenever possible, the GC–MS of synthetic compounds were analysed under the same conditions. An ext. standard was used for quantification; 0.5, 1.5 and 10 μg of synthetic octanoic acid were used with three replications. Integrated areas of peaks corresponding to either the ext. standard or each of the identified compounds were compared. Only compounds which represented more than 0.03% of the mixt. were considered.

Acknowledgements—Profs J. R. David and J. M. Jallon are thanked for their stimulating discussions and comments on the manuscript.

REFERENCES

1. Lecomte, M. H. (1924) *Flora Générale Indo-China; Rubiaceae* (vol. 3), p. 423. Pitard, J. and Masson et Cie, Paris.
2. Stones, B. C. (1970) *Micronesica* **6**, 551.
3. Degener, O. (1930) *Honolulu Star Bulletin*, p. 282.
4. Rock, J. F. (1974) *The Indigenous Trees of the Hawaiian Islands*, Charles E. Tuttle Company, p. 467.
5. Krishna Moorty, N. and Shama Reddy, G. (1970) *The Antiseptic* **67**, 167.
6. Bushnell, O. A., Fukuda, M. and Makinodan, T. (1950) *Pacif. Sci.* **4**, 167.
7. Adewunmi, C. O. and Kayode Adesogan, E. (1984) *Fitoterapia* **55**, 259.
8. Youngken, H. W. (1958) *J. Am. Pharmacol. Assoc.* **47**, 162.
9. Youngken, H. W., Jenkins, H. J. and Butler, C. (1960) *J. Am. Pharmacol. Assoc.* **49**, 271.
10. Levand, O. and Larson, H. O. (1976) *Planta Med.* **36**, 186.
11. Briggs, L. H. and Nicholls, G. A. (1954) *J. Chem. Soc.*, 3940.
12. Briggs, L. H. and Cain, B. F. (1954) *J. Chem. Soc.*, 4182.
13. Grimshaw, J. (1961) *Chem. Ind.* **1**, 403.
14. Briggs, L. H., Cain, B. F., Le Quesne, P. W. and Shoolery, J. N. (1965) *J. Chem. Soc.*, 2595.
15. Uddin Ahmad, V. and Bano, S. (1980) *J. Chem. Soc. Pakistan* **2**, 71.
16. Inoue, K., Nayeshiro, H., Inouye, H. and Zenk, M. (1981) *Phytochemistry* **20**, 1693.
17. Balakrishna, S., Seshadri, T. R. and Venkataramani, B. (1961) *J. Sci. Indust. Res. (India)* **20B**, 331.
18. Bowie, J. H. and Cooke, R. G. (1962) *Sp. Ed. J. Chem. Soc.*, 332.
19. Tsacas, L. and Bächli, G. (1981) *Rev. Fr. Entomol. (NS)* **3**, 146.
20. Louis, J. and David, J. R. (1986) *Acta Oecologica/Oecol. Gener.* **7**, 215.
21. Cariou, M. L. (1987) *Genet. Res.* **50**, 181.
22. R'Kha, S., Capy, P., David, J. R. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1835.
23. Legal, L., David, J. R. and Jallon, J. M. (1992) *Chemoecol.* **3**, 125.
24. Legal, L., Chappe, B. and Jallon, J. M. (1994) *J. Chem. Ecol.* **20**, 1931.
25. Higa, I. and Fuyama, Y. (1993) *Genetica* **88**, 129.
26. Moreteau, B., R'Kha, S. and David, J. R. (1994) *Behav. Genetics* **24**, 433.
27. Harborne, J. B. (1982) *Introduction to Ecological Biochemistry*, p. 227. Academic Press, London.
28. Banthorpe, D. V. and Brown, G. D. (1989) *Phytochemistry* **28**, 3003.
29. Zobel, A. M., Wang, J., March, R. E. and Brown, S. A. (1991) *J. Chem. Ecol.* **17**, 1859.
30. Zobel, A. M., Brown, S. A. and Nighswander, J. E. (1991) *Ann. Bot.* **167**, 213.
31. Zeringue, H. J. (1992) *Phytochemistry* **31**, 2305.
32. Marquardt, R. P. and Luce, E. N. (1961) *J. Agric. Food Chem.* **9**, 266.