Phytochemistry, 1975, Vol. 14, pp. 2013–2015. Pergamon Press. Printed in England.

SESQUITERPENE HYDROCARBONS OF FIJIAN GUAVAS

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(*Received* 14 *December* 1974)

Key Word Index—*Psidium guajava*; Myrtaceae; guava; essential oils; sesquiterpenes; chemotaxonomy.

Abstract—Wild guava trees (*Psidium guajava*) in Fiji can be classified into three main chemotypes on the basis of the relative amounts of sesquiterpene hydrocarbons present in the leaf essential oils. The principal components include caryophyllene, β-bisabolene, aromadendrene, β-selinene, nerolidiol, caryophyllene oxide and sel-11-en-4α-ol.

INTRODUCTION

The guava tree (*Psidium guajava*) is a native of Central America but has now spread throughout the tropics, where it is grown commercially for its fruit. It was first introduced in Fiji in 1863 when "Chilean guava" was sent from the Melbourne Botanical Gardens, subsequent introductions of unknown origin were made in the 1870s and 1880s [1]. Since then the tree has spread extensively and is now a declared noxious weed [2] and a widely used folkmedicine in the treatment of diarrhoea [3]. For a period wild fruit was used in jam manufacture and recently cultivation trials have been started using the commercial variety Beaumont.

The present work describes a study of the leaf essential oils of wild trees in Fiji as a guide to identifying chemical varieties. Other Myrtaceae, notably the genus Eucalyptus, have been reported to contain distinct chemovars on terpene analysis and it was of interest to see whether variations existed in Fiji in view of the probable diversity of the introduced stock or whether extensive hybridization has occurred. A limited amount of previous work has been carried out on the essential oils of guava leaves, although a number of studies have examined volatile flavour components of the fruit [4]. The leaves of wild and cultivated plants in the Argentine were reported to yield cineole and benzaldehyde [5] and two varieties from Allahabad [6] and Bangalore [7,8] in India were reported to yield limonene, caryophyllene, and an unidentified sesquiterpene and sesquiterpenoid alcohols. A recent study, using GLC, of the oil from trees in the Philippines did not, however, report any definite identifications [9].

During other chemical studies of guava trees, particularly of the composition of the fruit, variations in fruit shape, flesh colour and taste have been noted and in some cases differences have been found between cultivated and unidentified wild trees [10], but no systematic study of wild or cultivated trees has been reported.

RESULTS AND DISCUSSION

Examination of the low boiling constituents of the essential oil obtained by the steam distillation of the leaves of a guava tree, which produced sweet fruit, showed the presence of cineole and limonene as the major monoterpene components. In contrast, a tree with sour fruit yielded only a much smaller quantity of an unresolved mixture. In both cases benzaldehyde was also present but these components clearly represented a small proportion of the total oil.

GLC analysis of the sesquiterpene fractions showed that although both contained caryophyllene the other major constituents of the two trees were markedly different. The analysis was then

extended to a total of 47 wild trees, chosen from an extensive random collection to represent a wide range of morphological types, from south eastern Viti Levu, the main island in the Fiji group. Three different sesquiterpene patterns were recognized (Table 1) characterized by the presence of either β-bisabolene (**1**), β-selinene (**2**) or aromadendrene (**3**), respectively, in addition to caryophyllene. Most of the trees could be assigned to a distinct type but five trees were apparently hybrids and showed a combination of the characteristic patterns of two groups. This small proportion of hybrids is interesting as different chemotypes were often found in close proximity. A single example of "Chinese Guava", which is characterized by a yellow flesh in the fruit, unlike the normal pink or purple flesh, was also examined, and this gave a unique oil containing caryophyllene as the only significant sesquiterpene hydrocarbon.

The botanical characteristics of the trees were compared but no correlation could be found between chemotype and leaf size, shape or texture, bark colour or fruit shape, skin texture or flesh colour. Only the taste of mature fruit showed any relationship with chemical composition, Chemotype **2** being typically sweet and Chemotype **3** being typically sour. Chemotype **1**, however, showed a wide range of fruit taste from sweet to sour (Table 1). Attempts to correlate the fruit taste of this group with the composition suggested that proportionally large amounts of β-bisabolene characterized sweet fruit (sweet relative mean amount 186, intermediate 155, and sour 115) but in each case the range of values observed was large. In all analyses the leaves were taken from mature brown stems, as a comparison had shown that leaves from new green stems were almost identical but lacked some of the oxygenated compounds. Steam distillations or light petroleum extractions were employed to isolate the essential oils and the only major difference was the absence of benzaldehyde in the latter. The patterns of hydrocarbons and oxygenated compounds were the same irrespective of the method but the proportion of oxygenated sesquiterpenes was lower in extracted oils.

Two trees, types **2** and **3** and about 20 m apart, were analysed at intervals over 14 months. The steam distillates were compared and in both cases the pattern was unchanged but there were minor variations in the ratios of the components and both trees showed a slight relative reduction in caryophyllene content over the period. Although guavas in Fiji have a main season in May–July, they flower and fruit all year round.

The principal components of the essential oils were isolated from the oil obtained by steam distillation, by distillation, column chromatography and TLC. The individual compounds (Table 1) were then identified by IR, UV, PMR and MS. None of the components isolated correspond to

Table 1. GLC analysis of leaf essential oils from wild guava trees in Fiji

	Chemotype Relative R_t†		Mean peak areas relative to caryophyllene (range in parentheses for hydrocarbons)*					
MS200/12500	XE-60	Compound	**1**	**2**	**3**	"Chinese"	**1 & 3**	**2 & 3**
0·82	0·65	longicyclene	9(0–74)	2(0–6)	3(0–5)	2	3	1
1·00	1·00	caryophyllene	100	100	100	100	100	100
1·07	1·00	aromadendrene	‡		55(31–74)		10(6–17)	32(27–35)
1·14	1·29	(unidentified hydrocarbon)	62(34–125)	6(0–8)			47(38–57)	
1·29	1·17	β-bisabolene	251(126–420)		6(0–12)	32	189(184–195)	
1·30	1·38	β-selinene		132(81–342)				150(170–160)
1·53	2·82	nerolidiol	<1	15	4·5	40		2
1·86	4·23	caryophyllene oxide	6	15	24	18	4	12
2·40	4·68	(unidentified alcohol)	52				6	
2·41	6·31	sel-11-en-4α-ol		34	20	8		8
Number of plants examined			28	9	4	1	2	3
Fruit taste		Sweet	13	6	0	1	0	2
		Intermediate	7	2	0	0	2	1
		Sour	8	1	4	0	0	0

* The range is not given for oxygenated compounds as the values are partly dependent on the method of obtaining the extract.

† Relative to caryophyllene (*ca* 4·3 min on 8% MS200/12500 at 170°) and (*ca* 3·4 min on 2·5% XE-60 at 145°).

‡ When no values are given the compound was absent or present in insufficient amount for identification.

the unidentified $C_{15}H_{24}O$ alcohol isolated by Bhati from Allahabad guavas in India [8].

The leaves of the commercial variety Beaumont undergoing trial cultivation in Fiji were also examined and the essential oil showed a characteristic Chemotype 1 pattern with a high proportion of β-bisabolene. Thus this group of wild guavas could have a common origin with the cultivated variety. However, the determination of the geographical origin of all three chemotypes in Fiji will have to await future work on native guavas in other parts of the world, particularly South America.

These studies therefore provide a method for classifying guava trees based on their essential oils, which may be of assistance in breeding and hybridization studies and studies of plant migrations.

EXPERIMENTAL

Voucher specimens from a typical tree of each major chemotype are deposited in the Fiji Herbarium (Voucher nos. 18498–18503). The plants were identified by Mr. A. Sundaressen, and were collected during August 1972–August 1973 from Suva, Wailoku, Sawani and the Waimanu Valley, S.E. Viti Levu, Fiji.

Extraction of essential oils. (a) Minced guava leaves (*ca* 100 g) were steam dist. and distillate was extracted with Et_2O, after the addition of NaCl. Evaporation of the solvent gave a colourless oil (0·1–0·2% yield), which was used in the isolation of the major components. (b) For screening, the minced guava leaves were extracted with petrol (40–60°) at 25° for 18 hr and the extract analysed directly by GLC.

GLC analysis. Analyses were carried out on an FID instrument using glass columns 2 m × 3 mm id packed with either 8% Silicone fluid MS200/12500 on Chromosorb W at 170° or 2·5% XE-60 on Chromosorb G at 145°.

Identification of components. (a) Benzaldehyde, limonene, 1,8-cineole, caryophyllene, and longicyclene were identified by comparison with authentic samples on both GLC columns. (b) Essential oils from the steam distillation of related trees were combined and separated by chromatography on Si gel to give a hydrocarbon fraction after elution with petrol, and an oxygenated fraction after elution with $CHCl_3$. Both fractions were further fractionated by TLC on Si gel to give the individual compounds. In each case the PMR, IR, and MS agreed with the proposed structure.

Aromadendrene. The PMR spectrum correspond to an authentic spectrum [11].

β-Selinene. The PMR spectrum agreed with the lit. [12] and the R_t on both GLC columns was identical to the major component of celery seed oil [13].

β-Bisabolene. The MS [14] and PMR spectrum (CCl_4) δ 1·61 (3H), 1·67 (3H), 2·03 (3H), 4·68 (2H), 5·06 (1H), 5·30 (1H), agreed with the proposed structure but the PMR spectra differed from that reported earlier [15]. The compound had the same R_t as the major rearrangement product of nerolidiol [16].

trans-trans-*Nerolidiol.* This component had the same IR and GLC R_t as an authentic sample and its PMR spectrum agreed with that reported in ref. [17].

Sel-11-en-4α-ol. Identified by comparison with the IR and PMR spectra of an authentic sample [18].

Caryophyllene oxide. Identified by comparison with a sample prepared from caryophyllene [19,20].

Acknowledgements—The authors thank the Chemical Society Research Fund for financial assistance, Mr. R. W. Rickards of the Australian National University for measuring PMR and MS spectra, Dr. Enzell, Swedish Tobacco Company, for authentic sesquiterpene hydrocarbons, Professor Buchi of the Massachusetts Institute of Technology and Dr. Zalkow of the Georgia Institute of Technology for PMR spectra of authentic samples, and the Department of Agriculture, Fiji, for botanical specimens.

REFERENCES

1. (a) Parham, J. W. (1940–44) *Trans. Fiji Soc.* **2,** 76; (b) *The Journals of R. S. Swanston,* 1857–1866 (unpublished).
2. Mune, T. L. and Parham, J. W. (1967) *Declared Noxious Weeds of Fiji and Their Control, Fiji Department of Ag. Bull. No. 48,* p. 32, Fiji Government, Suva.
3. Sterly, J. (1970) *Heilflanzen der Einwohner Melanesiens,* p. 213. Kommissions Verlag Klaus Renner, Munich.
4. Stevens, K. L., Brekke, J. E. and Stern, D. J. (1970) *J. Agr. Food Chem.* **18,** 598.
5. Fester, G., Retamar, J., Ricciardi, A. and Romero-Fonseca, L. (1959) *Revista de la Facultad de Ingenieria Quimica Universidad Nacional del Litoral, Santa Fe* **28,** 9.
6. Bhati, A. (1953) *Perfumery Essent. Oil Record* **44,** 46.
7. Bhati, A., Bhattacharyya, S. C. and Guha, P. C. (1953) *Perfumery Essent. Oil Record* **44,** 274.
8. Bhati, A. (1967) *Perfumery Essent. Oil Record* **58,** 707.
9. Oliveros-Belardo, L., Cardeno, V. and Perez, P. (1971) *Flavour Ind.* **2,** 305.
10. e.g. see Chan, H. T., Brekke, J. E. and Chang, T. (1971) *J. Food Sci.* **36,** 237.
11. Büchi, G., Hofheinz, W. and Paukstelis, J. V. (1969) *J. Am. Chem. Soc.* **91,** 6473.
12. Bawdekar, A. S. and Kelkar, G. R. (1965) *Tetrahedron* **21,** 1521.
13. Schimmel & Co. (1910) *Chem. Zentr.* **I,** 1719.
14. Moshonas, M. G. and Lund, E. D. (1970) *Flavour Ind.* **1,** 375.
15. Manjarrez, A. and Guzman, A. (1966) *J. Org. Chem.* **31,** 348.
16. Anderson, N. H. and Syrdal, D. P. (1972) *Tetrahedron Letters* 2455.
17. Korthals, H.-P., Merkel, D. and Mühlstädt, M. (1971) *Ann. Chem.* **745,** 39.
18. Chetty, G. L., Zalkow, V. B. and Zalkow, L. H. (1968) *Tetrahedron Letters* 3223; Corbett, R. E. and Smith, R. A. J. (1967) *Tetrahedron Letters* 1009.
19. Damodaran, N. P. and Dev, S. (1968) *Tetrahedron* **24,** 4113, 4123; Thappa, R. K., Vashisht, V. N., Singh, J. and Sharma, R. K. (1970) *Curr. Sci. (India)* **8,** 182.
20. Treibs, W. (1947) *Chem. Ber.* **80,** 56.