A STUDY OF THE TERPENES AND STEROLS IN BLACK TEA BY THIN LAYER CHROMATOGRAPHY

A. S. L. Tirimanna and R. L. Wickremasinghe

The chemical compounds in the unsaponifiable fraction of black tea include the carotenoids, terpenes and sterols. This investigation deals with the separation and identification of some stable terpenes and sterols. Our interest in these compounds stems from the possibility that there may be a correlation between some of these compounds and tea flavour. Thin layer chromatographic methods were used for the separation. β-amyrin, linalool, phytol and spinasterol were found to be present in the tea extracts. The presence of other compounds too was detected but these have not been unequivocally identified.

Introduction

The unsaponifiable fraction of black tea extracts includes the carotenoids, terpenes and the sterols, all of which have a similar biosynthetic pathway, and possibly contribute directly or indirectly to the flavour of tea. The results of investigations on the carotenoids have been reported in a previous paper (Tirimanna & Wickremasinghe 1965) and this paper gives the results of studies on the terpenes and sterols.

The terpenes are odoriferous compounds, usually found in fragrant plants and are of great importance in the perfume industry. The sterols are a group of crystalline alcohols which include many pharmacologically active compounds of importance in the drug industry. Thin layer chromatographic methods were used and although less sensitive than gas chromatography, a number of compounds were separated. Some of these were found to be stable on storage whilst others were unstable. The present paper deals with the identification of four of the stable compounds.

Materials and methods

The black teas used in this study were obtained from sixteen estates in the Uva district during the period July and August 1965.

Extraction

The extraction procedure of the terpenes and sterols is essentially the same as that outlined for the carotenoids of the tea leaf (Tirimanna & Wickremasinghe 1965). The method used for the chemical isolation of sterols is described later.

Thin layer chromatography

Shandon TLC equipment was used. The thickness of the silica gel (Merck Darmstadt, Germany) layer on the glass plate was 0.25 mm. The solvent benzene-methanol (10 : 1) was used for the separation of the terpenes (Nigem *et al* 1965) while the solvent Ligroin (BP 100°-120°)—benzene—ethyl acetate (75 : 10 : 15) was used for the separation of the sterols (Rowe 1965). The silica gel plate was activated for 30 minutes at 110° before use.

Spray reagent

The presence of compounds on the silica gel plate was detected mainly by the anisaldehyde-conc sulphuric acid colour reagent of McSweeney (1965). This reagent was preferred to the commonly used vanillin-conc sulphuric acid as a wider range of colours were obtained with the various compounds present in the black tea extracts.

THE TERPENES

The terpenes usually contain fewer than 40 carbon atoms, and this distinguishes them from the group of carotenoids. However, both these classes of compounds (and also the sterols) are composed of isoprenoid units. The terpenes are broadly classified according to the number of carbon atoms in the compounds.

1 — Monoterpenes (C_{10}) *eg* geraniol, linalool, citronellol
2 — Sesquiterpenes (C_{15}) *eg* farnesol
3 — Diterpenes (C_{20}) *eg* phytol
4 — Triterpenes (C_{30}) *eg* -amyrin

All of the above with the exception of farnesol have been detected by different workers in tea, using gas chromatographic techniques. The thin layer chromatogram of an extract of black tea after saponification is represented in Figure 1.

Geraniol : A monoterpene alcohol reported to occur in green tea (Takei *et al* 1935 ; 1937) and in black tea (Takei *et al* 1938 ; Yamamoto *et al* 1940) although it could not be detected in the Uva teas analysed by the methods used here. It is commonly found in the oils of rose, palmarosa, citronella and lemon grass. It has a sweet rose odour.

Linalool : A monoterpene alcohol isomeric with geraniol and found to occur in small amounts in Uva teas (D in Figure 1). It has been reported to occur in green tea (Takei *et al* 1937) and in black tea (Yamamoto *et al* 1940). Yamanishi *et al* (1965) have also shown the occurrence of three isomers of linalool oxide in black tea.

Citronellol : A mixture of stereoisomeric monoterpene alcohols commonly found in the oils of geranium, rose and Java citronella. It has a sweet rose odour, and is reported to occur in black tea (Yamamoto & Kato 1934), although its presence was not detected in this study.

Farnesol : A sesquiterpene alcohol. Although it is of wide distribution, commonly found in the oils of citronella, lemon grass, rose and musk, it has not been reported to occur in tea.

Phytol : A diterpene alcohol formed as a decomposition product of chlorophyll. Its occurrence in black tea has been shown by thin layer chromatographic techniques.

β-amyrin : A pentacyclic triterpenoid compound. It is known to occur in the leaves of Canarium commune (Elemi bitter plant) and also in the latex of rubber as the acetate (Vesterberg & Westerlind 1922).

This compound (E in Figure 1) was detected in all the Uva black teas and identified by chemical reactions and also by comparison with an authentic marker. Quantitative variations in the concentrations were observed in the different black

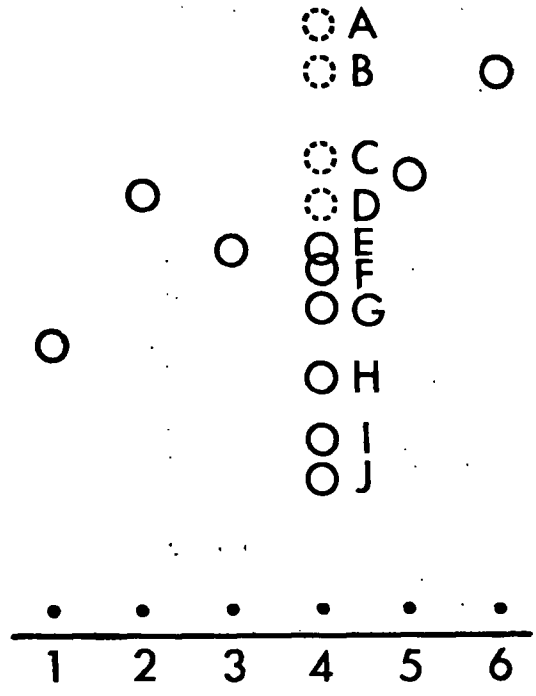

FIGURE 1—*Separation of terpenes —*
1 — Geraniol (.30); 2 — Linalool (.46); 3 — Farnesol (.40); 4 — Black Tea; 5 — Nerolidol (.48); 6 — Citronellol (.59); A — Unidentified (.64); B — Unidentified (.58); C — Unidentified (.50); D — Linalool (.45); E — β-amyrin (.40); F — Spinasterol (.38); G — Unidentified (.34); H — Unidentified (.26); I — Unidentified (.19); J — Lutein (.15); A dotted circle indicates a trace amount — The figures in brackets are the Rf values — The following colours were obtained with the anisaldehyde-conc sulphuric acid reagent: 1, 2, 3, 5, A, B, C, D — Greenish; 6 — Imperial purple; E — Purple; F — Blue; G — Reddish violet; H and I — Reddish brown — The solvent used was benzene-methanol (10: 1) v/v

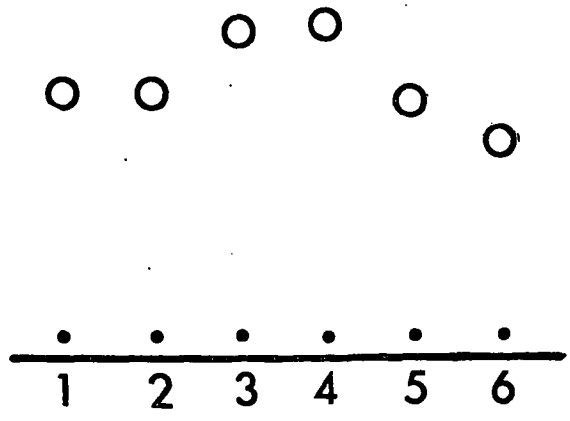

FIGURE 2—*Separation of sterols —*
1 — Cholesterol (.27); 2 — Stigmasterol (.27); 3 — Spinasterol in black tea after digitonin precipitation (.34); 4 — Spinasterol in Spinach leaves (.34); 5 — β-sitosterol (.26); 6 — Ergosterol (.22) — The figures in brackets are the Rf values — The solvent used was Ligroin-Benzene-Ethyl acetate (75-10-10) v/v/v

teas studied. This compound probably occurs in the tea leaf in a bound form as only trace amounts were detected before the saponification of the tea extract. β-amyrin was first isolated from green tea by Sakato (1942) and its presence confirmed by Ikeda (1943).

One of the stable compounds occurring in high concentration even before saponification gave many chemical reactions of β-amyrin, but differed from it in its Rf value (compound G in Figure 1). Accordingly, authentic markers of five compounds closely related to β-amyrin were co-chromatographed with the tea extract. The markers used were the triterpenoids, lupeol, taraxerol, betulin, morodiol and erythrodiol, but compound G differs in Rf value from all of these compounds.

The compounds A, B and C in Figure 1 were all unstable and disappeared on storage.

Two compounds (H & I in Figure 1) giving a characteristic reddish brown colour with the anisaldehyde reagent were detected in good quality teas. These compounds are very unstable during storage and are probably acidic as a yellow colour was obtained with the bromocresol green reagent. However, the triterpenoid acids, oleanolic (from cloves), ursolic (authentic marker), glycyrrhetinic (from liquorice root) and arjunolic (from *Terminalia arjuna*) did not correspond to either of these compounds.

THE STEROLS

The sterols are unsaponifiable compounds with pronounced pharmacological activity and characterised by precipitation with digitonin a rare glycoside obtained from the seeds of *Digitalis purpurea*. The sterols present in the tea extracts were precipitated with digitonin according to the method of Links, Verloop and Havinga (1961).

A solution of digitonin in 90% ethanol was added to the ethanolic plant extract in the proportion of 7 : 1 v/v. The suspension was left overnight in an atmosphere of nitrogen. The digitonides were first extracted with a solution of 90% ethanol and then with acetone. The combined extracts were evaporated to dryness under reduced pressure. The digitonides were taken up in a minimum amount of pyridine. A tenfold volume of peroxide free diethyl ether was added. It was left overnight and the ethereal solution containing the sterols were evaporated to dryness.

Using this method, followed by thin layer chromatograms only one sterol was found to be present in quantity in the tea extracts (Figure 2). Colour tests carried out on the silica gel plate confirmed that this compound was a sterol. A pink fluorescence was observed under ultra violet light after the plate was sprayed with 15% phosphoric acid and also with 25% trichloro-acetic acid in chloroform (Dawson *et al* 1959). Finally this compound was identified as spinasterol as it had an Rf value identical with that of the sterol of spinach leaves. There was no β-sitosterol (commonly found in higher plants), stigmasterol (found in soya and calabar beans) and ergosterol (found in yeast). This probably indicates that the tea plant is rather primitive among the higher plants but not so primitive as the pine which contains free cholesterol in the leaves (Rowe 1964).

Sakato (1942) isolated a sterol from the green leaves of tea and termed it theasterol and several years later Matsumoto *et al* (1955) isolated a sterol with the same melting point (166) and identified it as spinasterol. Itis a remarkably stable compound and persisted in black tea after eight months storage.

Summary

1— Thin layer chromatography techniques were developed for the separation of terpenes and sterols in black tea

2— Linalool (a monoterpene), phytol (a diterpene), β-amyrin (a triterpene) and spinasterol (a sterol) were found in extracts of black tea

3 — Five unidentified unstable compounds and one unidentified stable compound were also detected in the extracts

Acknowledgements

Our thanks are due to the District Advisory Officer, Passara, and to the Superintendents of the following estates for the samples of black tea, Aislaby, Battawatta, Craig, Dammeria, Delmar, Dyraaba, Glenanore, Glen Alpin, Mahadowa, Neluwa, St James, Serendib, Telbedde, Ury, Uva Highlands and Welimada ; to Mr D. J. M. Hettiarachi for the photograph ; to F. Hoffmann-La Roche & Co, Basle, Switzerland, for the generous gift of a sample of neriolidol ; to Dr J. Klinot, Charles University, Prague, Czechoslavakia for the samples of erythrodiol and morodiol; to Dr S. Huneck, Institute of Plant Chemistry, Dresden, Germany, for the sample of betulin ; to Dr Pasupati Sengupta, University of Kalyani, India, for the sample of taraxerol and to Drs R. O. B. Wijesekera and S. Sentheshanmuganathan, Medical Research Institute, Colombo, for the sample of β-amyrin, β-sitosterol, stigmasterol, cholesterol and ergosterol.

References

DAWSON, R. M. C. ; ELLIOT, D. C. ; ELLIOTT, W. H. & JONES, K. M. (1959). *'Data for Biochemical Research'*. Oxford, Clarendon Press. 263 pp.

IKEDA, H. (1943). Components of unsaponifiable matter of tea leaves. *J. Agr. Chem. Soc. Japan.* **19** : 301.

LINKS, J. ; VERLOOP, A. & HAVINGA, E. (1961). The steroids and higher fatty acids of the phytoflagellate Polytoma uvella. *Antonie Vanleeuwenhoek* **27** : 161-169.

MATSUMOTO, T. ; WAINAI, T. & MIYAKE, Y. (1955). Sterol of tea leaf. *Nippon Kagaku Zasshi.* **76** : 1057.

MCSWEENEY, G. P. (1965). Two methods for the thin layer chromatographic separation of terpene alcohols and identification by a colour reaction. *J. Chromatog.* **17** : 183-185.

NIGAM, M. C. ; NIGAM, I. C. & LEVI, L. (1965). Essential oil and their constituents XXV. Thin layer chromatography. Some chemical and chemotaxonomic applications. *J. Soc. Cos. Chem.* **16** : 155-168.

ROWE, J. W. (1965). The sterols of pine bark. *Phytochem.* **4** : 1-10.

SAKATO, Y. (1942). The chemical constituents of tea II Theosterol and β-amyrin. *J. Agr. Chem. Soc. Japan.* **18** : 524 .

TAKEI, S. ; SAKATO, Y. & ONO, M. (1935). Odiferous substances of green tea. VI. *Bull. Inst. Phy. Chem. Res. Tokyo.* **14** : 1262.

TAKEI, S. ; SAKATO, Y. & ONO, M. (1937). The essential oils of green tea VIII. *Bull. Inst. Phy. Chem. Res. Tokyo.* **16** : 7016.

TAKEI, S. ; SAKATO, Y. & ONO, M. (1938). Odiferous substances of green tea XI. Primary alcohols from oil of black tea. *Bull. Inst. Phy. Chem. Res. Tokyo.* **17** : 871.

TIRIMANNA, A. S. L. & WICKREMASINGHE, R. L. (1965). Studies on the quality and flavour of tea. 2. Carotenoids. *Tea Quart.* **36** : 115-121.

VESTERBERG & WESTERLIND (1922). *Ann.* **428**: 247. *Quoted Merck Index.* 7th Edition. 76.

YAMAMOTO, R. ; ITO, K. & TIN, H. (1940). Essential oil of Formosan black tea. *J. Agr. Chem. Soc. Japan.* **16** : 781.

YAMAMOTO, R. & KATO, A. (1934). Odour of black tea I. Odour of the leaves of black tea produced in Formosa during fermentation. *J. Agr. Chem. Soc. Japan.* **10** : 661.

YAMANISHI, T. ; KOBAYASHI, A. ; SATO, H. ; OHMURA, A. & NAKAMURA, H. (1965). the flavour of black tea. Part II. Alcohols and carbonyl compounds. *J. Agr. Biol. Chem.* **29**: 1016-1020.