Recent studies on the aroma of black tea have indicated that mono- and sesqui-terpenes are very important for the aroma of black tea. A survey of the literature reveals no work on the biosynthesis of these terpenoids in tea plant, although the knowledge of the biosynthetic mechanism is important in order to improve the quality of black tea. Therefore we began an investigation to elucidate the pathway of terpenoid biosynthesis in tea plant. A radioisotopic experiment was undertaken to determine whether or not acetate was incorporated into these terpenoids in tea plant.

Seven young shoots (15 g) of a tea plant, variety Benifuji, were excised and incubated with sodium acetate-2-¹⁴C (specific activity, 45.0 µCi/µmole, see Table I) in a closed chamber at 30±1°C under continuous illumination at intensity of approximately 10,000 lux. Air was circulated and passed through a trap containing 20% potassium hydroxide outside the chamber to absorb carbon dioxide. At the end of incubation, the shoots were homogenized with 120 ml of chloroform-methanol mixture (1:1) containing 30 mg each of linalool, geraniol, nerol, farnesol, and nerolidol as carriers, which are, except farnesol, main terpenoid components in tea plant. The homogenate was filtered and 50 ml of deionized water was added to the filtrate, and the mixture was separated by centrifugation. Radioactivity of each fraction was measured with a Packard Tri-Garb liquid scintillation spectrometer, model 314E.

Table I shows that 8.8% and 5.8% of the total radioactivity administered was present in the chloroform layer after incubation of the shoots for 2 and 4 days, respectively.

The chloroform layer of Expt. 1 in Table I was concentrated under reduced pressure at...
Below 35°C, and chromatographed on a silica gel column (1.7 x 30 cm, 23 g) by elution with stepwise increasing of ethyl acetate concentration in n-hexane. Percentage shown in Fig. 1 indicates volume concentration of ethyl acetate in n-hexane. Fractions of 10 ml each were collected and the radioactivity of an aliquot of each fraction was measured. The results are shown in Fig. 1; A, B, C, D and E correspond, respectively, to the elution sites of nerolidol, linalool, farnesol, nerol, and geraniol added as carriers, which were determined by color reaction with the anisaldehyde reagent.3) Among the fractions corresponding to these terpenoids the fraction A had the strongest radioactivity.

These fractions were further separated by means of thin-layer chromatography. After thin-layer chromatography of the fractions B, C and D, no radioactivity was detected in the zones of Rf values corresponding to authentic linalool, farnesol and nerol, respectively. Approximately 50% of the radioactivity in the fractions A and E was in the zones of Rf value corresponding to authentic nerolidol and geraniol, respectively. Results from thin-layer chromatography of the fraction A (nerolidol fraction) are summarized in Fig. 2.

Crystallization of derivatives was carried out in order to obtain further evidence for the synthesis of radioactive terpenoids. Four

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hundred mg unlabelled nerolidol was added as a carrier to the nerolidol fraction obtained column-chromatographically from the chloroform layer in Expt. 2 of Table I. The total activity used was 52,000 dpm. The nerolidol fraction was purified by thin-layer chromatography as described in Fig. 2, and then the radioactive nerolidol fraction was oxidized with chromic acid mixture into farnesal according to the method as described by M. Stoll.\(^4\) Crude farnesal was purified by silica gel column chromatography, and then, farnesal semicarbazone was prepared by the reaction of farnesal and semicarbazide-HCl in pyridine and ethanol solution. The product was repeated recrystallized from dilute ethanol. As shown in Table II, the specific activity of farnesal semicarbazone became constant during recrystallizations.

Geraniol 3-nitrophthalate was prepared by the method of Lennartz\(^5\) and recrystallization was repeated from cyclohexane. A constant specific radioactivity was not obtained with the repeated recrystallization, nevertheless a constant melting point was observed.

In preliminary examinations the following observations were made on column chromatography of the nerolidol fraction: young excised tea shoots were able to synthesize more nerolidol fraction than were unrooted tea seedlings (3 or 4 leaves, 4 months from sowing); radioactive nerolidol fraction was scarcely detected under the dark conditions, but it was produced under the alternating light and dark conditions, in the similar way to the case under continuous light.

From the results we have concluded that radioactive acetate is incorporated into nerolidol but not into linalool, nerol, geraniol and farnesol under the experimental conditions described above.

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