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ISOLATION AND STRUCTURE ELUCIDATION OF THEASPIRONE, A COMPONENT OF TEA ESSENTIAL OIL

Kazuo Ina and Yajiro Sakato
Department of Agricultural Chemistry, Shizuoka University
Iwata, Shizuoka-Ken
Hiroshi Fukami
Pesticide Research Institute, College of Agriculture
Kyoto University, Kyoto

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Extensive studies on volatile components of tea have been carried out by many authors and more than hundred compounds have been isolated, but any compound which plays a principal role on tea aroma have never been identified. Recently Bricout *et al.* have reported the isolation of 2-hydroxy-2,6,6-trimethyl-cyclohexylidene-1-acetic acid lactone as well as several new compounds from black tea as tea aroma plinciples[1]. In this communication we wish to report the isolation of theaspirone and the above cited lactone from tea essential oil as important components of tea aroma. Evidences for their structure elucidation are also presented.

The essential oil was extracted with methanol from black tea and separated into four fractions by preparative gaschromatography as shown in Fig. 1. The fraction C was separated by treatment with the Girard-T reagent into the carbonyl and the carbonyl free fraction, both of which has a strong odor of tea.

On preparative thin layer chromatography (silica gel 0.5 mm in thickness, *n*-hexane-ether 1:1) of the carbonyl fraction, the component showing R_f-value 0.4 - 0.5 was collected and repeatedly purified on tlc plate to give a colorless oil, which was named as theaspirone (yield 15 mg. from 1,000 Kg. of black tea). The presence of -CO-CH=C(CH$_3$)- grouping in theaspirone was confirmed by the following spectral data; UV, λ_{max}^{EtOH} = 234 $m\mu$, (ε = 16,000),

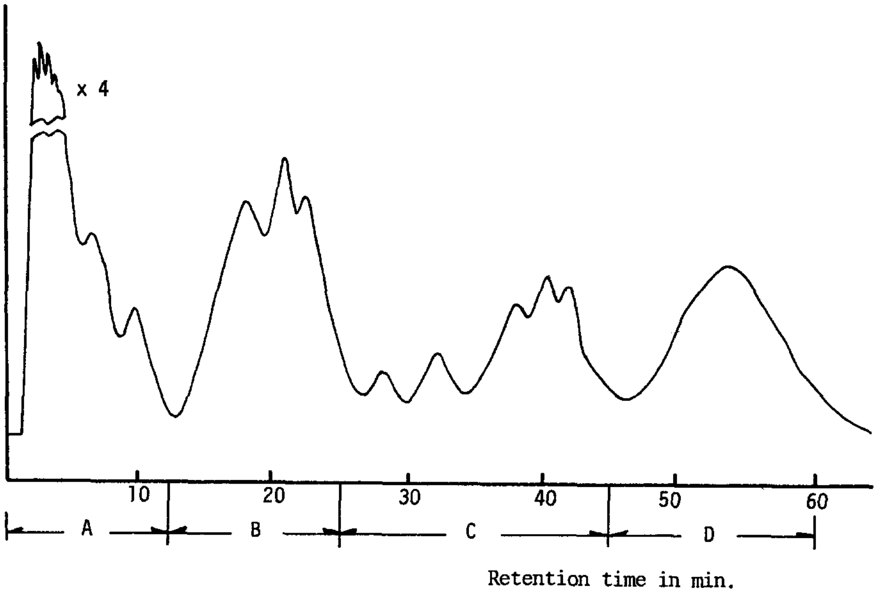

Fig. 1. Gas Chromatogram of the Neutral Fraction of the Essential Oil from Black Tea [with a column (200 cm x 14 mm) packed with 25% PEG 20M (Polyethyleneglycol) on celite 545 (30 - 60 mesh) at 180°C under nitrogen (flow-rate 1.5 l/min.)]

IR, 1660 cm^{-1}, nmr*, τ 8.03 (3H, doublet, J = 1.0 c.p.s.) coupling with a signal at τ 4.27 (1H, quartet)**. An absorption band at 1100 cm^{-1} in the IR spectrum implied an ether linkage, and the following nmr data suggested for the ether linkage to be contained in the grouping -C-O-CH(CH$_3$)-; a signal at τ 8.72 (3H, doublet, J = 6.1 c.p.s.)** coupling with an one proton signal (multiplet, centered at τ 5.9). The absence of hydroxyl group in the molecule was confirmed by the IR spectrum. The mass spectral parent peak was found at 208 mass units. It is likely from these data that the molecular formula of theaspirone is $C_{13}H_{20}O_2$. The nmr data indicated, besides the groupings mentioned above, *gem*-dimethyl (τ 9.08, 8.90, each 3H singlet) and six methylene protons (in a range between τ 8.5 and 7.5). On comparison of the nmr data with those of abscisin II[2] it is concluded that theaspirone has 4-hydroxy-isophorone ring moiety, being supported by the mass spectrum of theaspirone, which shows a strong peak at 152 mass units (M$^+$ - 56) as a result of loss of isobutylene fragment from the molecular

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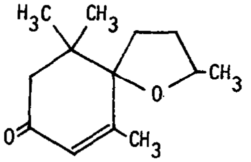
* The nmr spectra were measured with Varian A-60 or JNM-4H-60 in deuterio-chloroform with tetramethylsilane as internal reference. Grateful acknowledgement for these measurements is hereby made to Dr. T. Shingu, Department of Pharmacy, Kyoto University, and Mr. T. Ono, Tokyo College of Pharmacy.

** These couplings were confirmed by spin decoupling.

ion. The remaining groupings, $-CH_2CH_2-$ and $-O-CH(CH_3)-$, cooperate with a carbon atom of the isophorone ring to make up 2-methyl-tetrahydrofuran ring as shown in the formula I, being compatible with facts that the one proton signal at τ 4.27 is a sharp quartet, implying no hydrogen atom at the allylic position except hydrogens of the methyl group, and the chemical shift of the one proton multiplet around τ 5.9 coincides with that of 2-methyl-tetrahydrofuran itself (τ 6.1). Consequently theaspirone is to be assigned as 1-oxa-8-oxo-2,6,10,10-tetramethyl-spiro[4,5]-6-decene (I).



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The carbonyl free fraction was also separated into 12 fractions on tlc plate according to same procedure as mentioned above. From the fraction showing R_f-value 0.5 - 0.6 a colorless crystalline compound (m.p. 40 - 41°C) was obtained (20 mg. from 1,000 Kg. of black tea). The mass spectrum of this compound (M^+ 180) suggests its molecular formula to be $C_{11}H_{16}O_2$. The nmr spectrum indicates the presence of following groups in the molecule; three tertiary methyl groups (τ 8.78, 8.73, and 8.46, 3H singlet each), three methylene groups (6H signals in a range between τ 8.3 and 7.4), and β,β-disubstituted α,β-unsaturated γ-lactone moiety (α-vinyl proton, τ 4.37, 1H singlet), which is supported by IR data [1745 cm^{-1}(C=O), 1635 cm^{-1}(C=C)][3], and UV absorption [λ_{max}^{EtOH} 213 mμ (ε = 14,000)][3]. It is concluded from these spectral findings that the compound is assigned to be 2-hydroxy-2,6,6-trimethyl-cyclohexylidene-1-acetic acid lactone. This lactone has already been isolated by Sakan *et al.*[4] from *Actinidia polygama* and named as dihydroactinidiolide, and recently also isolated by Bricout *et al.*[1] from black tea aroma. Our lactone*, isolated as described above, was identical with dihydroactinidiolide** on the basis of superimposable nmr spectra.

A very diluted solution of these two compounds in their appropriate mixing ratio has fragrant odor of tea. Studies on identification of other several compounds, which are found in the fraction C (see Fig. 1) and seem to play an important role on tea aroma, are now in progress.

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* The optical rotatory measurement has not been made yet.

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