Cyclization of **4b** with 48% HBr-AcOH (2:1) gave α -2-benzyl-1',3',5,9-tetramethyl-thieno[4,3-*f*]morphan (**5b**) in 60% yield as colorless oil. **5b** HBr, mp 240—243° (decomp.) (ethanol-acetone), *Anal.* Calcd. for $C_{21}H_{27}NS \cdot HBr \cdot 1/2H_2O$: C, 61.81; H, 6.84; N, 3.28. Found: C, 62.22; H, 6.94; N, 3.31.

In the Table I, the signal for the 9-methyl group in **5a** or **5b**, respectively, appears as a diamagnetically shifted doublet, δ 0.85 or 0.79 ($J=7.0$ Hz) due to a position above the thiophene ring. Thus, the configuration of **5** was assessed as the α -diastereomer⁴⁾ in which both methyl groups in C₅- and C₉-positions were in a *cis* orientation.

The synthetic and biological studies of **5** and related compounds will be published in detail elsewhere in near future.

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Stereochemistry in Oxidation of Primary Allylic Alcohols by Cell-free System of Callus induced from *Cannabis sativa* L.

The pro-R hydrogen from C-1 methylene of primary allylic alcohols as geraniol and *trans*-cinnamyl alcohol was abstracted in cell-free system of callus induced from *Cannabis sativa* L.

We have previously reported¹⁾ that tetrahydrocannabinol, the other cannabinoids and essential oil which observed in the extract of original plant were not detected in the callus induced from *Cannabis sativa* L. (Moraceae). Then we have found that geraniol and nerol which are cannabinoids' precursors²⁾ were converted into citrals by the suspension cultures from Cannabis callus.³⁾ Further, without the addition of nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP), the activity in oxidation of primary and secondary allylic alcohols, that is, geraniol, nerol, *trans*-cinnamyl alcohol, (+)-*trans*-verbenol and (–)-isophorol, was demonstrated by the cell-free system from Cannabis callus.³⁾

In this paper, we report on the stereochemistry which is related to distinguish C-1 enantiomeric hydrogens of primary allylic alcohols.

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(*S*)-*trans*-Cinnamyl alcohol-1-D (I) and (*S*)-geraniol-1-D (IV) were prepared by reduction of *trans*-cinnamaldehyde and citral-a⁴) with isobornyloxymagnesium-2-D bromide according to the method of Streitwieser.⁵⁾ The deuterium contents of prepared alcohols were respectively over 98% from the proton magnetic resonance (PMR) spectra. Without the addition of NAD or NADP, the prepared alcohols-1-D were respectively incubated with the cell-free system (homogenized with 1 ml of Tris-buffer/1 g of fresh callus; 0.2M, pH 7.2) of callus induced from *C. sativa* L.¹⁾ for 4 hr at 26°. The biotransformed aldehydes were chromatographically purified from the *n*-hexane extracts of incubated filtrate. We have found that the obtained aldehydes consist of the mixtures of *trans*- and *cis*-compounds (84% of *trans*- and 16% of *cis*-cinnamaldehyde; 64% of citral-a and 36% of citral-b) from the PMR spectra and gas-liquid chromatography (GLC). On the other hand, a small amount of nerol, geometrical isomer of geraniol, was detected by GLC-MS of the extract in the conversion of geraniol. The PMR spectra (CDCl₃, δ , ppm) of aldehydes were as follows, *trans*-cinnamaldehyde; 6.63 (dd, $J=15.5$, 7.5 Hz, $\text{C}=\text{C}\langle\begin{smallmatrix} \text{CHO} \\ \text{H} \end{smallmatrix}\rangle$ and d, t, $J=15.5$, 2 Hz, $\text{C}=\text{C}\langle\begin{smallmatrix} \text{CDO} \\ \text{H} \end{smallmatrix}\rangle$, 7.42 (d, $J=15.5$ Hz, $\begin{smallmatrix} \text{H} \\ \text{Ph} \end{smallmatrix}\rangle\text{C}=\text{C}$), 7.43 (aromatic protons), 9.63 (d, $J=7.5$ Hz, CHO), *cis*-cinnamaldehyde; 6.12 (dd, $J=12.0$, 8.0 Hz, $\text{C}=\text{C}\langle\begin{smallmatrix} \text{H} \\ \text{CHO} \end{smallmatrix}\rangle$ and d, t, $J=12.0$, 2 Hz, $\text{C}=\text{C}\langle\begin{smallmatrix} \text{H} \\ \text{CDO} \end{smallmatrix}\rangle$, 7.35 (d, $J=12.0$ Hz, $\begin{smallmatrix} \text{H} \\ \text{Ph} \end{smallmatrix}\rangle\text{C}=\text{C}$), 9.88 (d, $J=8.0$

TABLE I.

Products	Position of integral	Integral	Normal type and deuterium type	Ratio of presence (%)
<i>trans</i> -Cinnamaldehyde	aldehyde H	15.5	(II)	36.9
	C-2 olefinic H	42.0	(II')	63.1
<i>cis</i> -Cinnamaldehyde	aldehyde H	3.0	(III)	33.3
	C-2 olefinic H	9.0	(III')	66.7
Citral-a	aldehyde H	7.0	(V)	35.0
	C-2 olefinic H	20.0	(V')	65.0
Citral-b	aldehyde H	4.0	(VI)	34.8
	C-2 olefinic H	11.5	(VI')	65.2

4) Citral-a was prepared by oxidation (MnO₂) of geraniol according to the method of J. Attenburrow, *et al.*, *J. Chem. Soc.*, 1094 (1952).

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Hz, CHO), citral-a⁶⁾; 1.63, 1.70 (s, respectively, $\text{CH}_3\text{C}=\text{C}$), 2.18 (d, $J=1.5$ Hz, $\text{CH}_3\text{-C}=\text{C}$), 5.06 (br, m, $\text{MeC}=\text{C-H}$), 5.85 (a signal of hanging bell form which based on the mixture of doublet $\text{C}=\text{C}\langle\text{H}\text{CHO}$ and triplet $\text{C}=\text{C}\langle\text{H}\text{CDO}$), 9.95 (d, $J=8.0$ Hz, CHO), citral-b⁶⁾; 1.61, 1.70 (s, respectively, $\text{CH}_3\text{C}=\text{C}$), 1.97 (d, $J=1.5$ Hz, $\text{CH}_3\text{-C}=\text{C}$), 5.11 (br, m, $\text{MeC}=\text{C-H}$), 5.85 (a signal of hanging bell form which based on the mixture of doublet $\text{C}=\text{C}\langle\text{H}\text{CHO}$ and triplet $\text{C}=\text{C}\langle\text{H}\text{CDO}$), 9.86 (d, $J=8.0$ Hz, CHO). As shown in Table I, in the cinnamaldehydes, the value of integral based on the proton of aldehyde against the value of integral based on the C-2 olefinic proton as one standard proton was 36.9% in *trans*-form and 33.3% in *cis*-form. Consequently, 36.9% of cinnamaldehyde and 63.1% of cinnamaldehyde-1-D exist in *trans*-form, and 33.3% of cinnamaldehyde and 66.7% of cinnamaldehyde-1-D in *cis*-form. In a similar manner, it was clear that 35.0% of citral-a and 65.0% of citral-a-1-D in *trans*-form, and 34.8% of citral-b and 65.2% of citral-b-1-D in *cis*-form. The fact that in the biotransformed aldehydes, the compounds which were substituted by deuterium existed more than the normal compounds, have shown that the pro-R hydrogen from the C-1 methylene of primary allylic alcohols as geraniol and *trans*-cinnamyl alcohol is lost. These results coincide with the result that horse liver alcohol dehydrogenase abstracts the pro-R hydrogen from the C-1 methylene of geraniol,⁷⁾ but the fact that the biotransformed aldehydes were the mixtures of *trans*- and *cis*-compounds, and nerol was detected in the conversion of geraniol, are interesting problems concerning with the *trans-cis* isomerization of primary allylic alcohols.

On the other hand, in the conversion of citral-a and citral-b by the same conditions mentioned above, citral-a was converted to citral-b with 30% of yield and citral-b was converted to citral-a with 25% of yield with 10 mg of the aldehydes to 40 g of fresh callus, but the corresponding alcohols were not detected.⁸⁾

Recently, a few papers were reported on the stereochemistry in *trans-cis* isomerization.⁹⁾ Although the mechanism of isomerization of primary allylic alcohols has not been evidenced yet, Imai and Marumo^{9a)} speculated that the process may include intermediary enolic forms in the aldehyde. But it is also thought that *trans-cis* isomerization may base on the radical reaction. We want to evidence this hereafter.

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