Cyclization of **4b** with 48% HBr–AcOH (2:1) gave α-2-benzyl-1′,3′,5,9-tetramethylthieno[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-diastereomer[4] in which both methyl groups in C_5- and C_9-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.

Research Laboratory
Sanwa Kagaku Kenkyusho Co., Ltd.
1212 Gejo-cho, Kasugai, 486, Japan

Masatoshi Ban
Yutaka Baba
Kenji Miura
Yasuaki Kondo
Kenji Suzuki
Mikio Hori

Gifu College of Pharmacy
5-Higashi, Mitafora, Gifu, 502, Japan

Received March 22, 1976

4) S.E. Fullerton, E.L. May, and E.D. Becker, *J. Org. Chem.*, **27**, 2144 (1962).

[Chem. Pharm. Bull. 24(7)1681—1683(1976)]

UDC 547.36.04 : 576.8.098 : 542.98

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 reported[1] 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' precursors[2] 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.

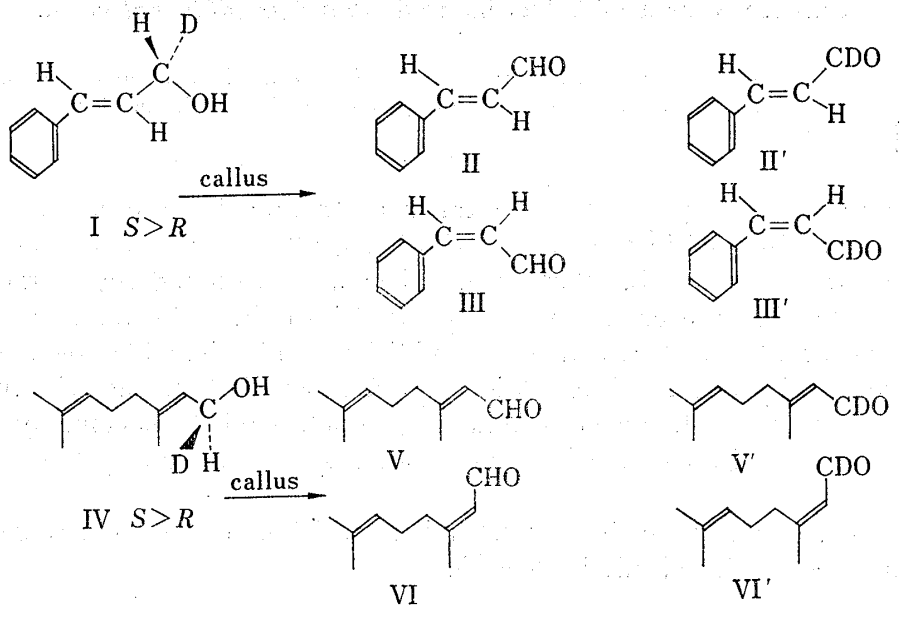
1) H. Itokawa, K. Takeya, and M. Akasu, *Shoyakugaku Zasshi*, **29**, 106 (1975).
2) *a*) R. Mechoulam and Y. Gaoni, *Tetrahedron*, **21**, 1223 (1965); *b*) Y. Shoyama, M. Yagi, and I. Nishioka, *Phytochemistry*, **14**, 2189 (1975).
3) Unpublished..

NII-Electronic Library Service

(*S*)-*trans*-Cinnamyl alcohol-1-D (I) and (*S*)-geraniol-1-D (IV) were prepared by reduction of *trans*-cinnamaldehyde and citral-a[4] 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 ($CDCl_3$, δ, ppm) of aldehydes were as follows, *trans*-cinnamaldehyde; 6.63 (dd, J=15.5, 7.5 Hz, C=C$\langle^{CHO}_{H}$ and d, t, J=15.5, 2 Hz, C=C$\langle^{CDO}_{H}$), 7.42 (d, J=15.5 Hz, $^{H}_{Ph}\rangle$C=C), 7.43 (aromatic protons), 9.63 (d, J=7.5 Hz, CHO), *cis*-cinnamaldehyde; 6.12 (dd, J=12.0, 8.0 Hz, C=C$\langle^{H}_{CHO}$ and d, t, J=12.0, 2 Hz, C=C$\langle^{H}_{CDO}$), 7.35 (d, J=12.0 Hz, $^{H}_{Ph}\rangle$C=C), 9.88 (d, J=8.0

Table I.

Products	Position of integral	Integral	Normal type and deuterium type	Ratio of presence (%)
trans-Cinnamaldehyde	aldehyde H	15.5	(II)	36.9
	C-2 olefinic H	42.0	(II′)	63.1
cis-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 (MnO_2) of geraniol according to the method of J. Attenburrow, *et al.*, *J. Chem. Soc.*, 1094 (1952).
5) A. Streitwieser Jr., J.R. Wolfe Jr., and W.D. Shaeffer, *Tetrahedron*, **6**, 338 (1959).

NII-Electronic Library Service

Hz, CHO), citral-a[6]; 1.63, 1.70 (s, respectively, $\begin{matrix}CH_3\\CH_3\end{matrix}\rangle C{=}C$), 2.18 (d, J=1.5 Hz, CH_3–C=C), 5.06 (br, m, $\begin{matrix}Me\\Me\end{matrix}\rangle C{=}C{-}H$), 5.85 (a signal of hanging bell form which based on the mixture of doublet $C{=}C\langle\begin{matrix}CHO\\H\end{matrix}$ and triplet $C{=}C\langle\begin{matrix}CDO\\H\end{matrix}$), 9.95 (d, J=8.0 Hz, CHO), citral-b[6]; 1.61, 1.70 (s, respectively, $\begin{matrix}CH_3\\CH_3\end{matrix}\rangle C{=}C$), 1.97 (d, J=1.5 Hz, CH_3–C=C), 5.11 (br, m, $\begin{matrix}Me\\Me\end{matrix}\rangle C{=}C{-}H$), 5.85 (a signal of hanging bell form which based on the mixture of doublet $C{=}C\langle\begin{matrix}H\\CHO\end{matrix}$ and triplet $C{=}C\langle\begin{matrix}H\\CDO\end{matrix}$), 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 ciral-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.

Tokyo College of Pharmacy
1432-1, Horinouchi,
Hachioji, Tokyo, Japan

Hideji Itokawa
Koichi Takeya
Michinori Akasu

Received April 19, 1976

6) *cf*) M. Ohtsuru, M. Teraoka, K. Tori, and K. Takeda, *J. Chem. Soc.* (*B*), **1967** 1033.
7) C. Donniger and G. Ryback, *Biochem. J.*, **91**, 11 (1964).
8) S. Marumo, *et al.*[9a] reported that farnesal was nonenzymatically isomerized into *trans* and *cis* mixture with the addition of albumin. In our experiments, citral was not isomerized with only Tris-buffer (0.2M, pH 7.2), but isomerized with cell-free system of callus which was deactivated with autoclave (2 atm, 5 min). The dry callus (about 2—5% of fresh callus) contains about 0.8% of protein according to the semi-micro Kjeldahl method.
9) *a*) K. Imai and S. Marumo, *Tetrahedron Letters*, 4401 (1974); *b*) K.H. Overton and F.M. Roberts, *Chem. Comm.*, **1975** 385; *c*) R. Evans and J.R. Hanson, *Chem. Comm.*, **1975** 231; *d*) B. Müller and C. Tamm, *Helv. Chim. Acta*, **58**, 483 (1975).

NII-Electronic Library Service