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Structural characterization of polyprenols by ^{13}C-n.m.r. spectroscopy: Signal assignments of polyprenol homologues

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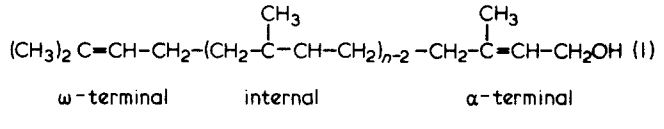
The ^{13}C-n.m.r. spectra of geraniol, nerol, four isomers of farnesol and eight isomers of geranylgeraniol were investigated as low molecular weight homologues of polyprenol. The aliphatic carbon signals were assigned by considering the correlation of chemical shifts between these compounds. The C-1 methylene carbona tom in *trans* units exhibited four signals around 39 ppm reflecting the *cis–trans*, *cis–trans*(α), *trans–trans* and *trans–trans*(α) linkages. The C-1 methylene carbon atom in *cis* units showed two signals around 32 ppm reflecting *cis–cis* and *cis–cis* linkages. Here, the ω terminal unit showed the same shielding effect as the *trans* unit on the subsequent unit. The geometric isomerism of the unit linked to the ω terminal unit was distinguished by the chemical shift of the C-1 methyl and C-4 methylene carbon signals. The geometric isomerism of the α terminal unit was determined from the chemical shift of the C-4 CH_2OH carbon signal. These correlations were independent of the molecular weight of polyprenol homologues.

Keywords Acyclic torpene; farnesol isomer; geranylgeraniol isomer; solanesol; geometric isomerism; carbon-13 nuclear magnetic resonance

INTRODUCTION

The occurrence of polyisoprenoid alcohols has been widely recognized in many higher plants, mammalian tissues and micro-organisms. These compounds are defined as a group of natural products whose carbon skeleton is made up of linear isoprene units linked head-to-tail. In the generalized structure (I), the degree of



polymerization *n* in polyprenols is reported to be between 6 and 24 depending on the source. The internal and α-terminal units exist in the *cis* or *trans* form. Most of the polyprenols consist of *cis* and *trans* units except for solanesol, which is recognized to be composed of the all-*trans* isoprene units. Ficaprenol-10 (C_{50}), -11 (C_{55}) and -12 (C_{60}), typical *cis-trans* polyprenols isolated from the leaves of *Ficus elastica*, were found to be composed of three internal *trans* units, five to seven internal *cis* units and one *cis* α-terminal unit[1]. Bacterialprenols and dolichols are another typical polyprenol with two internal *trans* units and several internal *cis* units[2]. The expected alignment of the *cis* and *trans* units was in the order; ω-terminal unit, three or two *trans* units, several *cis* units and *cis* α terminal units, which was estimated on the basis of the stereochemical observation of biosynthesis of beturaprenols[3]. However, there is no direct evidence to prove the location of the internal *cis* and *trans* units.

In previous work, we found a direct method to characterize alignment of *cis* and *trans* units by ^{13}C-n.m.r. spectroscopy[4]. This method is based on the fact that the C-1 methylene carbon atoms in isoprene units show four signals reflecting the *cis–trans*, *trans–trans*, *trans–cis* and *cis–cis* linkages in the case of *cis–trans* isomerized polyisoprenes[5].

Here, the ^{13}C-n.m.r. spectra of geraniol and nerol ($n=2$ in (I)), farnesol isomers ($n=3$) and geranylgeraniol isomers ($n=4$) were investigated as model compounds of polyprenol. The applicability of these signal assignments to polyprenols was discussed from the viewpoint of the dependence of chemical shifts on molecular weight of polyprenol homologues.

EXPERIMENTAL

Commercially obtained geraniol, nerol, *trans–trans* farnesol and solanesol were used without further purification. *Cis–trans*, *trans–cis* and *cis–cis* farnesol isomers were obtained by fractionation of a commercially produced synthetic farnesol with liquid chromatography[6]. Geranylgeraniol and geranylnerol were offered by Kuraray Co. Ltd. The other isomers of geranylgeraniol were prepared by isomerization of geranylgeraniol and geranylnerol with thiobenzoic acid under the irradiation of a high pressure mercury lamp and were separated by liquid chromatography[7].

The ^{13}C-n.m.r. spectrum was obtained using a JEOL

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FX-200 spectrometer at 50.1 MHz. Measurements were made at room temperature in $CDCl_3$ (about 5 w/v%) with tetramethylsilane as an internal standard. All the spectra were proton noise decoupled and obtained with multiple scans at a pulse repetition time of 7 s for a 45° pulse. Spectrum accumulation was carried out using a 16K computer for a spectral sweep width of 8000 Hz; the accuracy of the chemical shift was ±0.01 ppm.

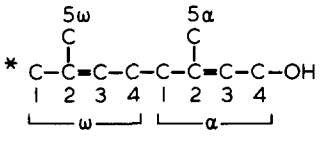
RESULTS AND DISCUSSION

Assignment of geraniol and nerol

The aliphatic carbon signals in geraniol and nerol were assigned by off-resonance measurements as shown in *Table 1*. The signals at 17.6 and 25.6 ppm were assigned to the methyl carbon atoms in the ω-terminal unit in *Z* and *E* configurations, respectively, which are designated as ω-*trans* and ω-*cis* in a similar way as the methyl carbon atom in *trans* and *cis* units. The assignment of geraniol is in good agreement with that by Jantret *et al.*[8].

Table 1 Assignment of aliphatic carbon signals in geraniol and nerol

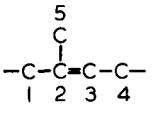
Chemical shift (ppm)				
Geraniol	Nerol	Assignment*		
59.41	59.02	4α	$-CH_2OH$	
39.60		1α	$-CH_2$	ω-*trans*
	32.05	1α		ω-*cis*
26.49	26.64	4ω	$-CH_2$	
25.65	25.63	1ω	$-CH_3$	*cis*
	23.40	5α	"	*cis*
17.69	17.65	5ω	"	*trans*
16.27		5α	"	*trans*



Assignment of farnesol isomers

The assignment of the aliphatic carbon atoms in four types of farnesol isomers was carried out by considering the relative intensity of the signals and the assignment of geraniol and nerol as shown in *Table 2*.

The methyl carbon atoms in the internal *cis* and *trans* units resonated at 16.0–16.3 and 23.3–23.4 ppm, respectively, which were differentiated from the signals due to the methyl carbon atoms in ω- and α-terminal units. The C–1 methylene carbon atoms in the *trans* and *cis* units resonated around 39 and 32 ppm, respectively, as in case of polyisoprene. Here, the carbon atoms are designated as follows:



The C–1 methylene carbon atom in the *cis* and *trans* units exhibited several signals reflecting the geometric isomerism of the unit linked to the C–1 carbon atom. The internal *trans* unit and ω-terminal unit caused a high-field shift on the C–1 methylene signal of the subsequent unit. The chemical shift of the C–1 methylene signal of the *trans* units was in the following order; *cis-trans*(α) > ω-*trans* > *trans-trans*(α). A similar tendency was observed for the C–1 methylene signals in the *cis* units, i.e., *cis-cis*(α) > ω-*cis* ≃ *trans-cis*(α).

The signals around 26 ppm were assigned to the C–4 methylene carbon atom in the internal and ω-terminal units by comparison of the chemical shifts of geranylgeraniol isomers.

Assignment of geranylgeraniol isomers

The aliphatic carbon signals in geranylgeraniol isomers were assigned as shown in *Table 3*. The C–1 methylene carbon atom in *trans* units resonated around 39.6 ppm

Table 2 Assignment of aliphatic carbon signals in farnesol iosmers

Chemical shift (ppm)						
trans–trans (α)	*trans–cis* (α)	*cis–cis* (α)	*cis–trans* (α)	Assignment*		
59.49	59.04	59.00	59.41	4α	$-CH_2OH$	
			39.85	1α	$-CH_2$	*cis–trans*(α)
39.74	39.72			1*m*	"	ω–*trans*
39.60				1α	"	*trans–trans*(α)
		32.34		1α	"	*cis–cis*(α)
	32.03	31.99	32.01	1*m*,α	"	ω–*cis*, *trans–cis*(α)
26.82	26.76			4ω	$-CH_2$	ω–*trans*
	26.61	26.70	26.62	4ω,*m*	"	ω–*cis*, *trans–cis*(α)
26.41		26.43		4*m*	"	*cis–cis*(α), *trans–trans*(α)
			26.25	4*m*	"	*cis–trans*(α)
25.67	25.67	25.67	25.69	1ω	$-CH_3$	*cis*
		23.43	23.35	5*m*	"	*cis*
	23.43	23.31		5α	"	*cis*
17.69	17.63	17.63	17.63	5ω	"	*trans*
16.29			16.27	5α	"	*trans*
16.04	16.02			5*m*	"	*trans*

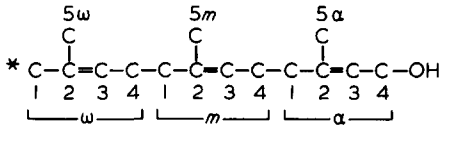
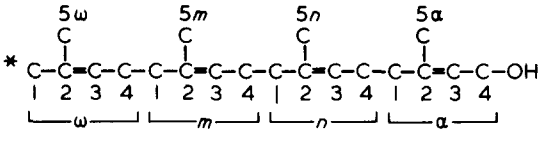


Table 3 Assignment of aliphatic carbon signals in geranylgeraniol isomers

Chemical shift (ppm)								Assignment*		
T–T–T$_\alpha$	*C–T–T*$_\alpha$	*T–C–T*$_\alpha$	*C–C–T*$_\alpha$	*T–T–C*$_\alpha$	*C–T–C*$_\alpha$	*T–C–T*$_\alpha$	*C–C–C*$_\alpha$			
59.43	59.39	59.43	59.47	59.08	59.06	59.06	59.08	4α	$-CH_2OH$	
	40.01				40.01			1*n*	$-CH_2$	*cis–trans*
		39.88	39.89					1α	"	*cis–trans*(α)
39.76		39.78		39.76 39.72		39.78		1*m,n*	"	ω–*trans*, *trans–trans*
39.60	39.58							1α	"	*trans–trans*(α)
			32.34			32.29	32.30	1*n*	"	*cis–cis*
	32.03	32.01	32.03	32.07	32.03	31.97	32.04	1*m,n,α*	"	*trans–cis*, ω–*cis*, *trans–cis*(α)
26.85				26.87				4ω	$-CH_2$	ω–*trans–trans*
		26.77	26.77			26.75	26.75	4ω	"	ω–*trans–cis*, ω–*cis–cis*
26.71	26.65			26.67	26.69			4ω,*m*	"	ω–*cis–trans*, *trans–trans*
		26.55		26.61	26.61	26.59		4*m,n*	"	*trans–cis*, *trans–cis*(α)
	26.57				26.53					*cis–trans*
26.40	26.36		26.46			26.36	26.44	4*m,n*	"	*trans–cis*(α)
										cis–cis(α), *cis–cis*
		26.24	26.30					4*n*	"	*cis–trans*(α)
	25.71		25.71		25.71		25.69	1ω	$-CH_3$	ω–*cis*
25.65		25.65		25.65		25.69		1ω	"	ω–*trans*
	23.39	23.36	23.42	23.41	23.37	23.45	23.35	5*m,n,α*	"	*cis*
17.67	17.63	17.67	17.65	17.67	17.63	17.69	17.63	5ω	"	*trans*
16.29	16.29	16.29	16.29					5α	"	*trans*
16.02	16.02	16.00		16.02	16.00	16.00		5*m,n*	"	*trans*



(*trans–trans*(α)), 39.7–39.8 ppm (ω-*trans* and *trans–trans*), 39.9 ppm (*cis–trans*(α)) and 40.0 ppm (*cis–trans*), while that in *cis* units resonated around 32.0–32.1 ppm (*trans–cis*, ω–*cis* and *trans–cis*(α)) and 32.3–32.4 ppm (*cis–cis* and *cis–cis*(α)). In this correlation it was observed that the ω-terminal unit has the same shielding effect as the internal *trans* unit on the subsequent C–1 methylene carbon atom. This is also the case for the farnesol isomers. This may be accounted for by the methyl group in *Z* configuration, which has a strong shielding effect on the subsequent C–1 methylene carbon atom to cause a high-field shift by 0.2–0.3 ppm. The shielding effect of the methyl group is supported by the fact that the C–1 methylene carbon atom of *cis* and *trans* units in polybutadiene resonated at 28.9 and 33.6 ppm, respectively, which show no splitting due to *cis–trans* linkages[9].

The C–1 methylene signals of the *trans* α-terminal unit showed a high-field shift by 0.10–0.15 ppm compared with that in the internal *trans* unit. However, the methyl signal of the *trans* α-terminal unit showed a low-field shift of 0.3 ppm. These phenomena were also observed in the *trans–trans*(α) of the farnesol isomer. This suggests that the hydroxyl group in the *trans* α-terminal unit is in a special conformation to exert a steric effect on the C–1 and methyl carbon atoms.

The C–1 methyl carbon atom in the ω-terminal unit showed a small difference in chemical shift reflecting the geometric isomerism of the subsequent unit, i.e., ω–*cis* at 25.72–25.74 ppm and ω–*trans* at 25.64–25.69 ppm. This tendency is not clear in the case of farnesol isomers, which may be due to the presence of a predominant effect of the α-terminal unit. The corresponding carbon atom in solanesol, which is the all-*trans* polyprenol with $n=9$ in (I), resonated at 25.66 ppm in agreement with the chemical shift of the ω–*trans* linkage.

The C–4 methylene signals around 26 ppm showed a very complicated splitting, reflecting the alignment of *cis* and *trans* units. The C–4 methylene carbon atoms in solanesol resonated at 26.83, 26.77 (main peak) and 26.38 ppm. The signal around 26.3–26.4 ppm was observed in farnesol and geranylgeraniol isomers having *cis–* and *trans–trans*(α) linkages. This indicates that the hydroxyl group in the trans α-terminal unit causes a high-field shift of 0.3 ppm on the C–4 methylene carbon atom linked to the α-terminal unit. the *cis–trans* isomerized polyisoprene showed the C–4 methylene signals at 26.77 (*trans–trans*), 26.70 (*trans–cis*), 26.56 (*cis–trans*) and 26.44 (*cis–cis*) ppm. The signals at 26.24–26.30 and 26.36–26.40 ppm were assigned to the *cis–trans*(α) and *trans–trans*(α) linkages, respectively, by considering the high-field shift of the corresponding signals in polyisoprene by 0.3 ppm. The signal corresponding to 26.83 ppm in solanesol was observed only for the isomers having the ω-*trans–trans* linkage. The other ω C–4 carbon atoms resonated at 26.65–26.77 ppm. This implies the presence of the shielding effect from the remote *trans* unit on the ω C–4 methylene carbon atom. The other C–4 methylene signals are tentatively assigned to the *cis* α-terminal units and *cis* and *trans* internal units by considering the correspondence of the chemical shifts of the farnesol and geranylgeraniol isomers.

Chemical shifts of polyprenol model compounds

The chemical shifts of aliphatic carbon atoms in polyprenol model compounds are plotted in *Figure 1*. Here, the chemical shifts are corrected by referring the ω methyl signal at 17.66 ppm as an internal standard. The chemical shifts of the aliphatic carbon signals are sensitive to the concentration of the sample in usual measurements using TMS as an internal standard. It can be compensated by using the common signal as the standard of chemical shift, though the correction is only ±0.03 ppm. The

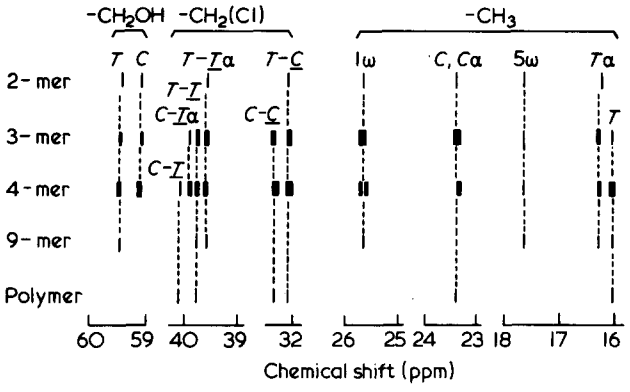
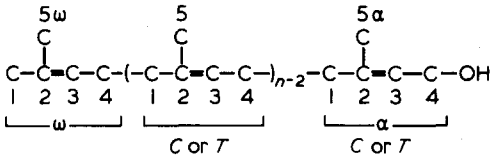


Figure 1 Chemical shifts of polyprenol model compounds. The carbon atoms are denoted as follows, where *T* and *C* correspond to *trans* and *cis* isoprene units:



chemical shifts of the signals in the *cis–trans* isomerized polyisoprene were uncorrected.

The C-4 CH_2OH carbon atom in *cis* and *trans* α-terminal units resonated at 59.0 and 59.4 ppm, respectively, independent of the molecular weight of the compounds.

The C-1 methylene carbon atom in *trans* units exhibited four signals reflecting the linkages of *cis–trans*, *cis–trans*(α), *trans–trans* (ω-*trans*) and *trans–trans*(α). The C-1 methylene carbon atom in *cis* units showed two signals due to *cis–cis* and *trans–cis* (ω-*cis*) linkages. Here, the *cis* α-terminal unit was indistinguishable from the internal *cis* unit.

The difference of the chemical shift in the ω C-1 carbon atom can be regarded as significant to distinguish the ω–*cis* and ω–*trans* linkages, though it is only 0.06–0.08 ppm. Similarly, the characteristic C-4 methylene signals at 26.24–26.30 (*cis–trans*(α), 26.36–26.40 (*trans–trans*(α)) and 26.57–26.77 ppm (ω-*trans–trans*) can be used to detect these linkages.

These findings indicate that the *cis–trans* linkages of the internal and α-terminal units and the geometric isomerism of the unit linked to the ω-terminal unit can be determined independently of the molecular weight of polyprenol homologues. The application of these assignments to the structural characterization of polyprenols will be presented in a subsequent paper.

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