Structure–Activity Relationships of a New Antifungal Imidazole, AFK-108, and Related Compounds

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Fungicidal activity of widely used imidazole antifungal drugs in topical applications is not so strong in spite of their potent fungistatic activities against dermatophytes and pathogenic yeasts. In order to improve fungicidal activity of imidazole antifungal agents, a series of novel imidazole derivatives having a hydrophobic substituent derived from isoprenoid were synthesized. The efficacy of these compounds was evaluated with respect to direct cell-membrane damaging activity, ergosterol biosynthesis inhibition, minimum growth-inhibitory concentration (MIC) and therapeutic effect for experimental dermatophytosis of guinea pigs. Among the newly synthesized compounds, the geranyl derivative named AFK-108 (2a) showed the highest *in vivo* fungicidal activity with both cell membrane damaging activity and ergosterol biosynthesis inhibition *in vitro*.

Key words imidazole; geraniol; antifungal activity; structure–activity relationship; AFK-108

Imidazole derivatives are widely used in topical antifungal chemotherapy because of their broad spectrum and high availability.[2)] It has been recognized that imidazole antifungals act with at least two distinct mechanisms. One is the inhibition of ergosterol biosynthesis at low concentration below 10^{-6} M, which is responsible for fungistatic action. The other is direct physicochemical cell membrane damage exerted at higher concentration between 10^{-5}—10^{-4} M (*i.e.*, 5—10 μg/ml), which causes the fungicidal effect.[3)] Since a high concentration is necessary for the latter effect, conventional imidazole antifungals do not act as fungicidal but fungistatic agents under therapeutic conditions. Although fungistatic drugs are effective for improving the condition of patients, recurrence of the condition is often observed after suspension of the application. Enhancement of the fungicidal activity of imidazole antifungal agents is expected to overcome such problem.

In this paper, we describe the design and synthesis of a series of novel imidazole compounds, which are expected to enhance fungicidal action, and the structure–activity relationships of these compounds.

Chemistry It has been reported that geraniol is readily incorporated into cell membranes of yeasts, such as *Candida* and *Saccharomyces* spp.[4)] Thus, we designed isoprenoid-substituted imidazoles by introducing various isoprenoids (or derivatives) into the 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethane skeleton, which is employed as the core structure of many existing azole antifungals. Though this skeleton has an asymmetric carbon, all the compounds were synthesized from the racemic 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethane skeleton, which is also used for conventional miconazole.

First of all, **2a** which has a geranyl side chain was synthesized because of the reason described above. The compounds **4**—**6** were synthesized in order to compare the activity of its geometrical isomer or its saturated compounds. The compounds **2b**—**d** were synthesized in order to see that the activity was maintained if the linkage oxygen atom was replaced by a sulfur or nitrogen atom.

The principal routes for synthesizing the designed compounds are outlined in Charts 1 and 2, and are described in detail in the experimental section.

1-(2,4-Dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethanol, 1-chloro-1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethane, 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethylamine and *N*-methyl-1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethylamine were synthesized by the method described before.[5)]

The introduction of isoprenoid substituents into this skeleton was carried out by the reaction of 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethanol or 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethylamine with halide prepared from the corresponding isoprenoidal alcohol. Monoolefinic derivative **5** was hydrogenated using Pd–C to give the saturated **6**, and both compounds were obtained as a mixture of diastereomers. Sulfur containing **2b** was obtained by the reaction of thiogeraniol and 1-chloro-1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethane.

All the compounds could be isolated as crystals or powders of the fumarates from ethanol.

Antifungal Activity The antifungal activity of the newly synthesized imidazole compounds was evaluated *in vitro* by the following criteria. There was a direct cell-membrane damaging effect, which was determined by using K^+ release from the treated cells as an index, inhibitory effects on ergosterol biosynthesis by the intact cells assumed from the incorporation of ^{14}C-acetate into cellular ergosterol, and minimum growth-inhibitory concentration (MIC). In addition, *in vivo* efficacy of these compounds was evaluated with the experimental dermatophytosis of guinea pigs. In these evaluations, clotrimazole and miconazole, which are conventional imidazole drugs, were used as the reference compounds. The results of the evaluation experiments are summarized in Table 1.

The MIC values against *Trichophyton* spp. and *Candida* spp. are shown in the 1st and 2nd columns, respectively, of Table 1. Among the newly synthesized compounds, prenyl derivative **1** showed the lowest MIC value for *Trichophyton* spp., and the MIC values increased in order of **2a** (geranyl

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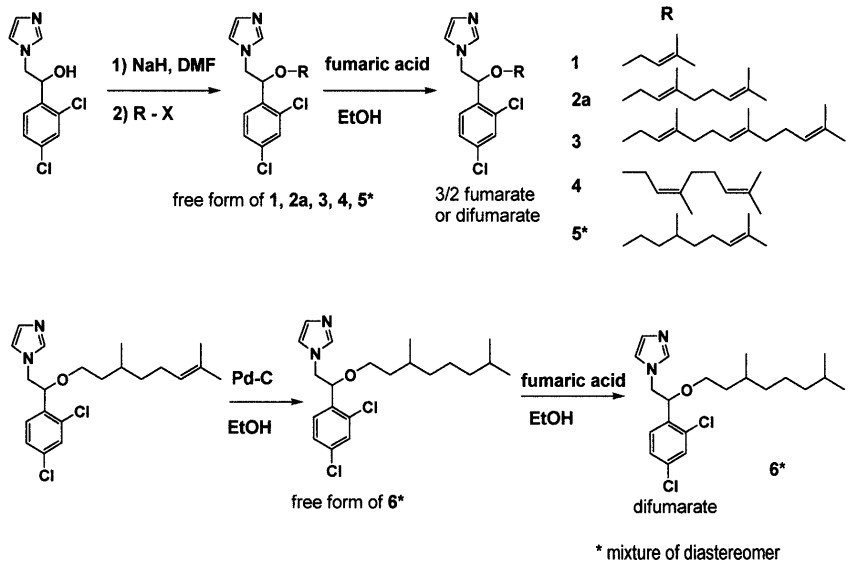

Chart 1

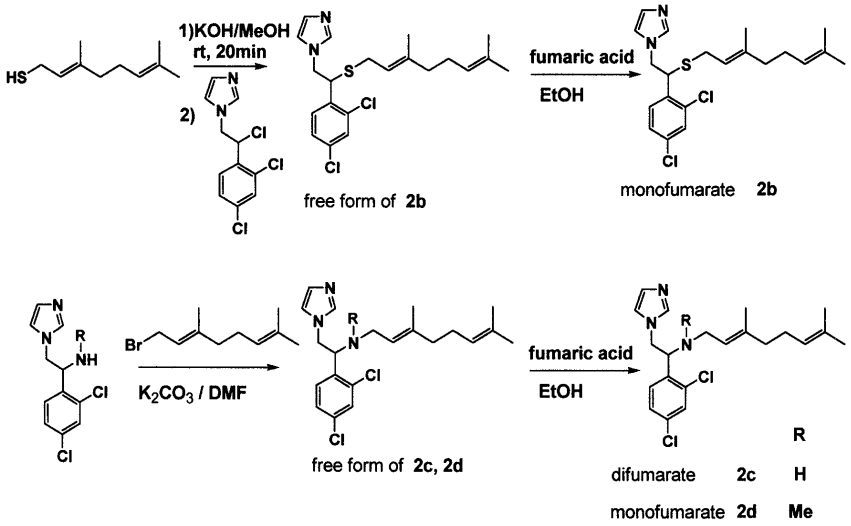

Chart 2

derivative)<**4** (geometrical isomer of **2a**)<**5** and **6** (saturated derivatives of **2a** with respect to the inner double bond)< **2b**—**d** (sulfur or nitrogen derivatives of **2a**)<**3** (farnesyl derivative). The MIC values of these compounds for *Candida* spp. were different. Lower MIC values were obtained for the compounds having geranyl or its derivatives, such as **2a**, **2c**, **2d**, **4**—**6**, and higher MIC values were observed on **1** having prenyl group and **3** having farnesyl group. Among geranyl-containing compounds, **2b** showed higher MIC value. It is also pointed out that none of these newly synthesized compounds showed a lower MIC than the reference compounds, clotrimazole and miconazole, except that the MIC value of **1** for *Trichophyton* spp. was comparable to those of the reference compounds.

Direct cell-membrane damaging activities of the newly synthesized imidazoles estimated by using *C. albicans* TIMM 0144 are summarized in the 4th column of Table 1. High damaging activity was observed on the compounds having geranyl or its derivatives, **2a**, **4**, **5** and **6**, and the highest one was **5** with the partially saturated geranyl moiety. However, the compounds having the nitrogen or sulfur linkage atoms between imidazole part and the geranyl substituent (**2b**—**d**) showed significantly lower activity. The membrane damaging activity of **3** bearing the longer farnesyl group was lower than that of **2a** and that of **1** having a shorter prenyl group was very low. Thus, it can be concluded that all synthesized compounds except **1** had fairly strong direct cell-membrane damaging activity against *C. albicans* TIMM 0144. This fact suggests that a certain length of the isoprenoid chain is necessary for the direct cell-membrane damaging activity. It is noteworthy that **2a**, **2c**, **2d**, **4**—**6** which had relatively low MIC against *C. albicans* showed high direct cell-membrane damaging activity and **1**, **3**, and **2b** which had high MIC showed low direct cell-membrane damaging activity. The direct cell-membrane damaging activities of the newly synthesized compounds were significantly higher than those of the reference imidazoles.

The inhibitory effects of the newly synthesized imidazoles on *in vitro* ergosterol biosynthesis by *C. albicans* TIMM 0144 are summarized in the 3rd column of Table 1. The in-

Table 1. Antifungal Activity of Isoprenoid Substituted Imidazoles (**1**, **2a—d**, **3—6**) and Conventional Imidazoles

	MIC (μg/ml)		Ergosterol biosynthesis inhibitory effect[a]	Direct cell-membrane damaging effect[b]	Experimental dermatophytosis in guinea pigs[c] Rate of nagive culture
	Trichophyton spp.	*Candida* spp.	$-\log ED_{50}$ (M)	K^+ release (%)	Mean±S.D. (%)
1	0.63	80—160	3.18	17.9	18.6±19.9
2a (AFK-108)	5	40	4.90	80.6	58.6±20.6
3	40—80	>80	3.24	54.5	25.7±22.4
4	5—10	20—40	4.08	73.8	54.3±23.5
5	10	20—40	3.81	86.2	27.1±25.7
6	10	20	2.36	79.2	21.4±17.7
2b	20	>80	4.00	46.8	37.1±17.7
2c	10—20	40	4.81	65.3	45.7±25.5
2d	10—20	40	3.91	64.8	33.6±17.9
Miconazole	0.63—1.25	10	4.84	36.9	27.1±16.8
Clotrimazole	0.16—0.31	1.25—10	3.75	4.9	24.2±18.1

a) *C. albicans* TIMM0144. *b*) *C. albicans* TIMM0144 (treated with 40 μg/ml). *c*) *T. mentagrophytes* TIMM1189 (treated with 1% in vaseline).

hibitory effects of **2a** and **2c** were 10 to 50 times higher than the others, and these were comparable to that of miconazole. Compounds **2a** and **2c** have a geranyl group linked with the ether and imino linkages, respectively, to the fundamental structure common in all of the newly synthesized compounds. Compound **4**, the geometrical isomer of **2a**, showed slightly weaker inhibition than **2a**. The inhibitory effect of compounds **2b** (thioether homologue of **2a**) and **2d** (*N*-methylated derivative of **2c**) were significantly lower than those of **2a** and **2c**. Substitution of the geranyl group of **2a** with either shorter prenyl **1** or longer farnesyl **3** chain reduced the inhibitory effect. Saturation of the double bond(s) of geranyl group (**5** and **6**) also decreased the potency of the inhibition. These results suggested the special importance of the geranyl group for inhibiting *in vivo* ergosterol biosynthesis by *C. albicans*.

The *in vivo* efficacy of the newly synthesized imidazoles was estimated by using the rate of negative culture in experimental dermatophytosis of guinea pigs with *Trichophyton mentagrophytes* TIMM 1189, and the result is shown in the last column of Table 1. The highest efficacy was obtained with **2a** having a geranyl group and **4**, the geometrical isomer of **2a**. The *in vivo* efficacy was followed by **2c** and **2d** with a nitrogen linkage atom replaced by the ether linkage atom of **2a**, and **2b** (thioether homologue of **2a**). However, the efficacy was markedly decreased in **5** and **6**, which were saturated at one or two double bonds of the geranyl group, and **1** and **3** having prenyl and farnesyl groups, respectively. The *in vivo* efficacy of the most potent **2a** was about two times higher compared to those of the reference compounds, miconazole and clotrimazole, estimated under the same conditions. The compounds showing high *in vivo* efficacy described above had a geranyl group, such as **2a**, and these compounds showed both marked inhibitory effects on ergosterol biosynthesis and potent direct cell-membrane damaging activity. This fact indicates that the imidazole compounds showing high activity in both of these two *in vitro* tests tend to have high *in vivo* efficacy. However, there was no correlation between the MIC values against *Trichophyton* spp. or *Candida* spp. and the *in vivo* efficacy determined by the experimental dermatophytosis.

Discussion

The newly synthesized compound **2a** having a geranyl group showed the most potent antifungal activity in both *in vitro* and *in vivo* evaluations. The inhibitory effects of the series of isoprenoid substituted imidazoles on yeast lanosterol 14α-demethylase ($P450_{14DM}$), the primary target of azole antifungal agents, has been studied in detail by Yoshida and his co-workers[6] with the purified enzyme preparation. The strongest inhibition was caused by compound **2a**, and the inhibitory effect was reduced when the geranyl group of **2a** was replaced by a longer farnesyl, **3**, or a shorter prenyl, **1**, groups. Saturation of two double bonds of the geranyl moiety of **2a** reduced the inhibitory effect on the activity of purified $P450_{14DM}$.[6] Such structure and activity relationships were held on the inhibitory effects of these imidazoles for the inhibition of the ergosterol biosynthesis by intact cells of *C. albicans* TIMM 0144 described here. Consequently, it is evident that the inhibitory effect of the newly synthesized imidazole compounds for the ergosterol biosynthesis by intact cells depends on their binding to $P450_{14DM}$, which is the primary target of azole antifungal agents.[7]

It is generally known that the activity of azole antifungal agents determined *in vitro*, such as inhibition of ergosterol biosynthesis and direct cell-membrane damaging activity, is poorly correlated with their MIC values. For example, clotrimazole shows fairly low MIC value, but it shows neither high $P450_{14DM}$ inhibitory activity nor high direct cell-membrane damaging activity. The MIC values of the compounds synthesized in this study against *Candida* spp. were also poorly correlated with these *in vitro* indices. Although a small correlation was observed between MIC values and the direct cell-membrane damaging activity, their MIC values could not be explained only by this activity.

The efficacy of the newly synthesized imidazoles assumed from the rate of negative culture in experimental dermatophytosis of guinea pigs with *Trichophyton* spp. was not associated with the MIC values for *Trichophyton* spp. The efficacy of these compounds was correlated with both the inhibitory effect of ergosterol synthesis and direct cell-membrane damaging activity against *Candida* spp. High efficacy was observed on **2a**, **4**, and **2c** that showed high activity in both of the two *in vitro* assays, whereas the compounds showing high direct cell-membrane damaging activity but

low $P450_{14DM}$ inhibitory activity, such as **5** and **6**, showed low efficacy. Such a tendency was also observed with the reference compounds, miconazole and clotrimazole. The efficacy of these compounds determined under the same conditions was low, although they showed rather high inhibitory effect on ergosterol synthesis. This may be due to their low direct cell membrane damaging activity. These findings suggest that both inhibitory effect on $P450_{14DM}$ and direct cell-membrane damaging activity are essential for high *in vivo* efficacy of the imidazole antifungal compounds, and the newly synthesized imidazoles having a geranyl moiety or its derivatives, such as **2a**, **4** and **2c**, are the compounds having such properties necessary for effective fungicidal compounds.

Conclusion

Among the newly synthesized imidazoles, AFK-108 (**2a**) bearing a geranyl moiety showed considerable high fungicidal activity in the *in vivo* efficacy test with experimental dermatophytosis. The potent fungicidal activity of this compound is dependent on both the inhibition of $P450_{14DM}$ and direct cell-membrane damaging activity. Substitution of the geranyl moiety with other isoprenoid group or partial modifications of the geranyl moiety decreased either or both of the inhibitory effect on $P450_{14DM}$ and direct cell-membrane damaging activity, and reduced the *in vivo* fungicidal activity. There was no correlation between the efficacy for experimental dermatophytosis and the MIC values. These results suggest that both direct cell-membrane damaging activity and ergosterol biosynthesis inhibition are more important than apparent MIC for fungicidal activity *in vivo*. Introduction of an isoprenoid chain with an appropriate length, such as a geranyl group, may be effective for improving fungicidal activity of imidazole compounds.

Experimental

Melting points were determined using a Büchi 535 melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a Hitachi 260-50 spectrometer. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken on a Bruker AC-200P (200 MHz) spectrometer with tetramethylsilane as an internal standard. Abbreviations used are as follows: s, singlet; d, doublet; m, multiplet; br, broad; br s, broad singlet; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide. Merck Kiesel gel 60 was employed for silica gel column chromatography.

1-[2-(2,4-Dichlorophenyl)-2-[(*E*)-3,7-dimethylocta-2,6-dienyloxy]-ethyl]-1*H*-imidazole 3/2Fumarate (2a) To a stirred solution of 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethanol (10.0 g, 39.0 mmol) in DMF (30 ml) was gradually added sodium hydride (1.56 g, 39.0 mmol) at room temperature. The reaction mixture was stirred at 40 °C for 30 min. After cooling to −15 °C, to the stirred reaction mixture was added (*E*)-1-bromo-3,7-dimethylocta-2,6-diene (2.60 g 12.0 mmol) in DMF and the reaction mixture was further stirred for 30 min at room temperature. After evaporation of solvent under reduced pressure, the residue was diluted with chloroform. The chloroform layer was washed with water and dried over anhydrous sodium sulfate. Concentration followed by column chromatography on silica gel (ethyl acetate : hexane = 1 : 3—1 : 2) gave 12.6 g (82%) of the free form of **2a** as a colorless oil. IR (neat): 2968, 2932, 2860, 1590, 1506, 1473, 1440, 1386, 1287, 1233, 1218, 1107, 1095, 1077, 1044, 1005, 909, 867, 825, 789, 756, 663, 627 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, s), 1.60 (3H, s), 1.69 (3H, s), 1.87—2.22 (4H, m), 3.79 (1H, dd, J=7.3, 11.7 Hz), 3.91 (1H, dd, J=6.6, 11.7 Hz), 3.99 (1H, dd, J=7.3, 14.3 Hz), 4.15 (1H, dd, J=2.7, 14.3 Hz), 5.03—5.12 (1H, m), 5.17—5.24 (1H, m), 6.93 (1H, s), 7.02 (1H, s), 7.22—7.38 (2H, m), 7.38—7.43 (1H, m), 7.48 (1H, s).

The obtained free amine (0.98 g, 2.5 mmol) was dissolved in ethanol (10 ml), and fumaric acid (0.43 g, 3.7 mmol) in ethanol (30 ml) was added. After removal of the ethanol under reduced pressure, a small amount of ether and then hexane were added to give white crystals. The crystals were collected by filtration and recrystallized from ethyl acetate–hexane (2 : 1). 0.97 g of **2a** (3/2fumarate) was obtained as white crystals. mp 105.7—106.4 °C. *Anal.* Calcd for $C_{21}H_{26}Cl_2N_2O \cdot 3/2$fumarate: C, 57.15; H, 5.68; N, 4.94. Found; C, 56.86; H, 5.68; N, 4.92. The ratio of free formed amine/fumaric acid was changed by the amount of fumaric acid and the conditions of recrystallization.

1-[2-(2,4-Dichlorophenyl)-2-(3-methyl-2-butenyloxy)ethyl]-1*H*-imidazole 3/2Fumarate (1) This compound was obtained from 4-bromo-2-methyl-2-butene by a procedure similar to that used for the preparation of **2a** from (*E*)-1-bromo-3,7-dimethylocta-2,6-diene. Free form of **1**: Colorless oil, yield 51%. IR (neat): 2980, 2936, 2880, 1592, 1506, 1472, 1442, 1384, 1288, 1232, 1108, 1090, 1046, 1010, 866, 824, 790, 664 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.51 (3H, s), 1.71 (3H, s), 3.75 (1H, dd, J=7.6, 11.5 Hz), 3.88 (1H, dd, J=6.8, 11.5 Hz), 3.99 (1H, dd, J=7.3, 14.4 Hz), 4.16 (1H, dd, J=2.9, 14.4 Hz), 4.91 (1H, dd, J=2.9, 7.3 Hz), 5.14—5.31 (1H, m), 6.93 (1H, s), 7.10 (1H, s), 7.28 (1H, d, J=1.7 Hz), 7.30 (1H, s), 7.41 (1H, d, J=1.7 Hz), 7.45 (1H, s). **1**: White crystals (acetone–hexane), mp 112.2—114.7 °C. *Anal.* Calcd for $C_{16}H_{18}Cl_2N_2O \cdot 3/2$fumarate: C, 52.92; H, 4.84; N, 5.61. Found: C, 52.73; H, 4.85; N, 5.69.

1-[2-(2,4-Dichlorophenyl)-2-((*E*,*E*)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)ethyl]-1*H*-imidazole Difumarate (3) This compound was obtained from (*E*,*E*)-1-chloro-3,7,11-trimethyldodeca-2,6,10-triene by a procedure similar to that used for the preparation of **2a** from (*E*)-1-bromo-3,7-dimethylocta-2,6-diene. Free form of **3**: Colorless oil, yield 25%. IR (neat): 2928, 2860, 1592, 1506, 1472, 1442, 1386, 1232, 1108, 1092, 1044, 824, 790, 662 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, s), 1.60 (6H, s), 1.68 (3H, s), 1.84—2.22 (8H, m), 3.78 (1H, dd, J=7.5, 11.6 Hz), 3.90 (1H, dd, J=6.5, 11.6 Hz), 3.98 (1H, dd, J=7.3, 14.4 Hz), 4.16 (1H, dd, J=2.8, 14.4 Hz), 4.91 (1H, dd, J=2.8, 7.3 Hz), 5.01—5.29 (3H, m), 6.93 (1H, s), 7.02 (1H, s), 7.26—7.32 (2H, m), 7.38—7.42 (1H, m), 7.45 (1H, s). **3**: White crystals (acetone–hexane), mp 79.1—81.2 °C. *Anal.* Calcd for $C_{26}H_{34}Cl_2N_2O \cdot$ difumarate:C, 58.87; H, 6.10; N, 4.04. Found: C, 58.47; H, 6.17; N, 4.16.

1-[2-(2,4-Dichlorophenyl)-2-((*Z*)-3,7-dimethylocta-2,6-dienyloxy)-ethyl]-1*H*-imidazole 3/2Fumarate (4) This compound was obtained from (*Z*)-1-bromo-3,7-dimethylocta-2,6-diene by a procedure similar to that used for the preparation of **2a** from (*E*)-1-bromo-3,7-dimethylocta-2,6-diene. Free form of **4**: Colorless oil, yield 36%. IR (neat): 2968, 2926, 2860, 1590, 1566, 1506, 1473, 1449, 1383, 1287, 1233, 1107, 1095, 1077, 1044, 1008, 867, 825, 789, 735, 663, 627 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, s), 1.64 (3H, s), 1.71 (3H, s), 1.82—2.10 (4H, m), 3.76 (1H, dd, J=7.3, 11.4 Hz), 3.89 (1H, dd, J=6.6, 11.4 Hz), 4.00 (1H, dd, J=7.3, 14.4 Hz), 4.17 (1H, dd, J=6.6, 14.4 Hz), 4.91 (1H, dd, J=2.9, 7.3 Hz), 4.98 (1H, m), 5.20 (1H, dd, J=6.6, 7.3 Hz), 6.93 (1H, s), 7.01 (1H, s), 7.20—7.39 (2H, m), 7.41 (1H, d, J=1.7 Hz), 7.45 (1H, s). **4**: White crystals (acetone–hexane), mp 86.9—88.7 °C. *Anal.* Calcd for $C_{21}H_{26}Cl_2N_2O \cdot 3/2$fumarate: C, 57.15; H, 5.68; N, 4.94. Found; C, 57.17; H, 5.68; N, 4.89.

1-[2-(2,4-Dichlorophenyl)-2-(3,7-dimethylocta-6-enyloxy)ethyl]-1*H*-imidazole 3/2Fumarate (5) This compound was obtained from (*S*)-citronellyl bromide by a procedure similar to that used for the preparation of **2a** from (*E*)-1-bromo-3,7-dimethylocta-2,6-diene. Free form of **5** was obtained as a diasteromer mixture: Colorless oil, yield 48%. IR (neat): 2964, 2928, 2876, 1592, 1564, 1506, 1472, 1440, 1384, 1286, 1232, 1098, 1078, 1044, 824, 790, 662 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, d, J=6.3 Hz), 1.60 (3H, s), 1.69 (3H, s), 1.04—1.80 (5H, m), 1.85—2.18 (2H, m), 3.18—3.49 (2H, m), 3.98 (1H, dd, J=7.1, 14.3 Hz), 4.17 (1H, dd, J=2.7, 14.3 Hz), 4.84 (1H, dd, J=2.7, 7.1 Hz), 5.01—5.20 (1H, m), 6.92 (1H, s), 7.01 (1H, s), 7.23—7.29 (2H, m), 7.40—7.47 (2H, m). **5**: White crystals (acetone–hexane), mp 99.7—100.2 °C. *Anal.* Calcd for $C_{21}H_{28}Cl_2N_2O \cdot 3/2$fumarate: C, 56.95; H, 6.02; N, 4.92. Found; C, 56.83; H, 6.07; N, 4.91.

1-[2-(2,4-Dichlorophenyl)-2-(3,7-dimethyloctyloxy)ethyl]-1*H*-imidazole Difumarate (6) Pd–C (0.11 g) was added to a solution of 1-[2-(2,4-dichlorophenyl)-2-(3,7-dimethylocta-6-enyloxy)ethyl]-1*H*-imidazole (1.1 g, 2.8 mmol) in ethanol (20 ml) at room temperature. The reaction mixture was stirred for 7 h under H_2 atmosphere. The reaction mixture was filtered through Celite and concentrated under reduced pressure, followed by column chromatography on silica gel (hexane) gave 0.59 g (52%) of the free form of **6** as a colorless oil. IR (neat): 2960, 2932, 2876, 1506, 1472, 1444, 1386, 1286, 1232, 1106, 1076, 1034, 758, 748, 662 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, d, J=6.2 Hz), 0.85 (3H, s), 0.88 (3H, s), 0.98—1.72 (10H, m), 3.16—3.48 (2H, m), 3.98 (1H, dd, J=7.3, 14.3 Hz), 4.18 (1H, dd, J=2.8, 14.3 Hz), 4.85 (1H, dd, J=2.8, 7.3 Hz), 6.96 (1H, s), 7.04 (1H, s), 7.20—7.43 (3H, m), 7.45 (1H, s). **6**: White crystals (pentane), mp 68.8—70.6 °C. *Anal.* Calcd for $C_{21}H_{30}Cl_2N_2O \cdot$ difumarate: C, 55.33; H, 6.08; N, 4.45. Found; C, 55.70; H, 6.10; N, 4.40.

1-[2-(2,4-Dichlorophenyl)-2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-

ethyl]-1*H*-imidazole Difumarate (2c) To a solution of 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethylamine (2.50 g, 9.8 mmol) in DMF (30 ml) was added potassium carbonate (1.35 g, 9.8 mmol) and (*E*)-1-bromo-3,7-dimethylocta-2,6-diene (2.12 g, 9.8 mmol) at room temperature. Stirring was continued for 30 min at room temperature. After evaporation of solvent under reduced pressure, the residue was diluted with ethyl acetate, washed with water, followed by drying over anhydrous sodium sulfate. Concentration followed by column chromatography on silica gel (chloroform : methanol=150 : 1) gave the free form of **2c** (1.08 g) as a colorless oil. IR (neat): 2968, 2928, 2856, 1590, 1506, 1470, 1440, 1386,1284, 1232, 1108, 1078, 1046, 1032, 864, 824, 788, 754, 662 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (1H, s), 1.44 (3H, s), 1.60 (3H, s), 1.68 (3H, s), 1.73—2.17 (4H, m), 2.99 (1H, dd, J=7.5, 13.6 Hz), 3.04 (1H, dd, J=6.6, 13.6 Hz), 3.97 (1H, dd, J=7.7, 14.0 Hz), 4.18 (1H, dd, J=4.2, 14.0 Hz), 4.51 (1H, dd, J=4.2, 7.7 Hz), 4.98—5.15 (2H, m), 6.87 (1H, s), 7.04 (1H, s), 7.23—7.45 (4H, m). The obtained oil was dissolved in ethanol (10 ml), and fumaric acid (1.08 g, 9.3 mmol) in ethanol was added. After removal of the ethanol, a small amount of ether and then hexane were added to give white crystals. The crystals were collected by filtration and recrystallized from acetone–hexane (2 : 1). 0.89 g of **2c** (difumarate) was obtained as white crystals, mp 116.5—118.0 °C. *Anal.* Calcd for $C_{21}H_{27}Cl_2N_3O$·difumarate: C, 55.77; H, 5.65; N, 6.73. Found; C, 55.69; H, 5.75; N, 6.74.

1-[2-(2,4-Dichlorophenyl)-2-[*N*-[(*E*)-3,7-dimethylocta-2,6-dienyl]-*N*-methylamino]ethyl]-1*H*-imidazole Monofumarate (2d) This compound was obtained from *N*-methyl-1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethylamine by a procedure similar to that used for the preparation of **2a** from 1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethylamine. Free form of **2d**: Colorless oil, yield 18%. IR (neat): 2972, 2924, 2860, 1590, 1508, 1472, 1454, 1386, 1234, 1108, 1080, 1050, 822, 664 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, s), 1.60 (3H, s), 1.68 (3H, s), 1.89—2.20 (4H, m), 2.31 (3H, s), 3.02 (2H, d, J=6.8 Hz), 4.12—4.40 (3H, m), 5.03—5.12 (1H, m), 6.70 (1H, s), 6.92 (1H, s), 7.14—7.25 (3H, m), 7.33 (1H, d, J=1.6 Hz) **2d**: White crystals (acetone–hexane), mp 136.0—136.7 °C. *Anal.* Calcd for $C_{22}H_{29}Cl_2N_3$·monofumarate: C, 59.77; H, 6.37; N, 8.04. Found; C, 59.65; H, 6.47; N, 8.08.

1-[2-(2,4-Dichlorophenyl)-2-[(*E*)-3,7-dimethylocta-2,6-dienylthio]-ethyl]-1*H*-imidazole Monofumarate (2b) To a stirred suspension of potassium ethyl dithiocarbonate (8.05 g, 50.2 mmol) in acetone (70 ml) was added (*E*)-1-bromo-3,7-dimethylocta-2,6-diene (10.9 g, 50.2 mmol) at room temperature. The reaction mixture was stirred for 30 min. After removal of the precipitate by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in ether, washed with water, dried with anhydrous sodium sulfate. After evaporation of the ether, to the residue was added ethylenediamine solution (70%, 15 ml) at 0 °C and the mixture was stirred for 1 h at room temperature. The reaction mixture was poured into dil. H_2SO_4/crushed ice and extracted with ether. The extract was washed with dil. H_2SO_4, dried with anhydrous sodium sulfate, and concentrated under reduced pressure. To a solution of 1.0 g (5.9 mmol) of the residue (9.87 g) in methanol (40 ml) was added potassium hydroxide (0.33 g, 5.8 mmol) and 1-chloro-1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)ethane (1.62 g, 5.9 mmol) in ethanol at room temperature, and then the reaction mixture was refluxed for 90 min. After cooling, the solvent was evaporated and then the residue was diluted with chloroform. The chloroform layer was washed with water, followed by drying over anhydrous sodium sulfate. Concentration followed by column chromatography on silica gel (ethyl acetate : hexane=2 : 3) gave the free form of **2b** (1.32 g) as a colorless oil. IR (neat): 2976, 2928, 2860, 1590, 1506, 1474, 1448, 1386, 1288, 1232, 1108, 1078, 1052, 866, 826, 784, 734, 662 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, s), 1.59 (3H, s), 1.67 (3H, s), 1.90—2.17 (4H, m), 3.01 (2H, d, J=8.0 Hz), 4.25 (2H, d, J=6.4 Hz), 4.60 (1H, t, J=6.4 Hz), 4.98—5.18 (2H, m), 6.77 (1H, s), 6.98 (1H, s), 7.25 (1H, dd, J=2.1, 8.4 Hz), 7.29 (1H, s), 7.37 (1H, d, J=2.1 Hz), 7.42 (1H, d, J=8.4 Hz) **2b**: White crystals (acetone–hexane), mp 130.8—132.2 °C. *Anal.* Calcd for $C_{21}H_{26}Cl_2N_2O_4S$·monofumarate: C, 57.14; H, 5.75; N, 5.33. Found; C, 57.05; H, 5.94; N, 5.26.

Antifungal Activity The fungistatic activity was investigated *in vitro* against isolates of *Trichophyton* spp. and *Candida* spp. Minimum inhibitory concentration (MIC) were determined using a conventional agar dilution method.[8]

Direct cell-membrane damaging effect of imidazole was assumed from the imidazole-dependent release of potassium from the growing cells. *C. albicans* TIMM 0144, which had been grown on Sabouraud's glucose broth for 4 weeks, was treated with an imidazole compound (40 μg/ml) at 37 °C for 15 min. Imidazoles were added to the growth medium as DMSO solution. The incubated suspension was filtered, and the potassium ion in the filtrates was determined with a Varian SpectraAA-40 atomic absorption spectrophotometer. Total potassium content of the cells was assumed from the potassium concentration in the hot 5% perchloric acid extracts of the imidazole-untreated cells.

Inhibitory effect of imidazoles on *in vivo* ergosterol biosynthesis by growing cells of *C. albicans* was examined as follows. The cells of *C. albicans* TIMM 0144 growing on Sabouraud's glucose broth were labeled with 1 μCi of sodium [^{14}C]acetate for 2 h in the presence of various concentrations of an imidazole compounds. The labeled cells were collected and lipids were extracted by the Bligh and Dyer's method.

The extracted lipids were separated by thin layer chromatography (TLC) on precoated Silica gel 60C plates (Merck no. 13142) using heptane–isopropyl ether–glacial acetic acid, 60 : 40 : 4 (v/v/v), as a solvent. Bands on the TLC plate was detected by a TLC-scanner (BIOSCAN System 200 imaging scanner) and each lipid was identified by comparison on TLC with standard.

Therapeutic effect of the imidazole compounds as antifungal agent was evaluated by topical treatment of experimental guinea pig dermatophytosis caused by *T. mentagrophytes* TIMM 1189 according to the method of Yamaguchi *et al.*,[9] with partial modification. The tests were carried out with 5—6 guinea pigs for each drug. Two locations of dorsal trunk were inoculated with a conidial suspension of *T. mentaprophytes* TIMM 1189. Topical treatment with 0.1 g of vaseline ointment of a test compound (1%) was started 5 d after the inoculation. The ointment was applied once a day for 6 d. Two days after the last treatment, the infected area of the skin was cut into small pieces, and each piece of skin was cultured on Sabouraud's dextrose plates at 27 °C for 14 d. The rate of negative cultures was calculated from the number of skin pieces without fungal growth and the total number of skin pieces.

References and Notes

1) Present address: *Department of Bioengineering, Faculty of Engineering, Soka University, Hachioji 192–0003, Japan.*
2) Georgiev V. S. (ed.), "Annals of the New York Academy of Sciences, Antifungal Drugs," Vol. 544, The New York Academy of Sciences, New York, 1988.
3) Beggs W. H., Hughes C. E., *Diagn. Microbiol. Infect. Dis.*, **6**, 1—3 (1987).
4) Bard M., Albrecht M. R., Gupta N., Guynn C. J., Stillwell W., *Lipids*, **23**, 534—538 (1988).
5) *a*) Godefroi E. F., Heeres J., Cutsem J. V., Janssen P. A. J., *J. Med. Chem.*, **12**, 784—791 (1969); *b*) Sandoz A.-G., Patent DE 3408127 (1984) [*Chem. Abstr.*, **102**, 62244 (1985)].
6) *a*) Aoyama Y., Ishida K., Hori K., Sakaguchi A., Kudoh M., Yoshida Y., *Biochem. Pharmacol.*, **44**, 1701—1705 (1992); *b*) Ito T., Aoyama Y., Ishida K., Kudoh M., Hori K., Tsuchiya S., Yoshida Y., *ibid.*, **48**, 1577—1582 (1994).
7) Van den Bossche H., "Current Topics in Medical Mycology," Vol. 1, ed. by Mcginnis M. R., Springer, New York, 1985, pp. 313—351.
8) Bligh E. G., Dyer W. J., *Can. J. Biochem. Phys.*, **37**, 911—917 (1959).
9) Yamaguchi H., Uchida K., *Dermatologica*, **169**, Suppl. 1, 33—46 (1984).