Optically Active Antifungal Azoles. V.[1] Synthesis and Antifungal Activity of Stereoisomers of 3-Azolyl-2-(substituted phenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanols

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The (2*S*,3*S*)-, (2*R*,3*S*)- and (2*S*,3*R*)-stereoisomers of (2*R*,3*R*)-3-azolyl-2-(substituted phenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanols [(2*R*,3*R*)-1a—d] were prepared and evaluated for antifungal activity against *Candida albicans in vitro* and *in vivo* to clarify the relationships between stereochemistry and biological activities. The results revealed that the *in vitro* antifungal activity in each set of the four stereoisomers [(2*R*,3*R*)-, (2*S*,3*S*)-, (2*R*,3*S*)- and (2*S*,3*R*)-1a—d] definitely paralleled the *in vivo* antifungal activity against candidosis in mice, and the order of potency was (2*R*,3*R*)≫(2*R*,3*S*)≥(2*S*,3*S*)≥(2*S*,3*R*).

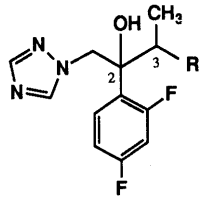
In addition, the four stereoisomers in each set were assessed for sterol biosynthesis-inhibitory activities in *C. albicans* and rat liver. The (2*R*,3*R*)-isomer was found to exert a strong and selective inhibitory effect on the sterol synthesis in *C. albicans* as compared with that in rat liver.

Key words optically active antifungal azole; stereoisomer; 1,3-bis(azolyl)-2-butanol; antifungal activity; cytochrome P450; sterol biosynthesis

Antifungal azoles are known to inhibit the biosynthesis of ergosterol, which is essential for maintaining the integrity of the fungal cell membrane. The crucial enzyme in the biosynthesis of ergosterol is lanosterol 14α-demethylase (cytochrome $P450_{14DM}$) which acts on the substrate, lanosterol, to remove the 14α-methyl group *via* oxidative cleavage.[2] The binding site for oxygen on this enzyme is blocked by azoles, and this leads to the depletion of ergosterol as well as the accumulation of 14α-methylsterols in the cell membrane, resulting in the inhibition of the growth of fungal cells. The structure–activity relationships seen with azoles suggest that the enzyme recognizes inhibitors in which the functional group has a specific arrangement, *i.e.*, azolyl, halophenyl, hydroxy (or ether) or lipophilic groups.[3] Furthermore, the stereoselective interaction of the enzyme with stereoisomeric azoles has become an intriguing field of study. The different affinities of a variety of P450's for the stereoisomers of ketoconazole have been reported.[4] In addition, the relationship between the enzyme interaction and antifungal activity has been studied with the stereoisomers of diniconazole[5] and triadimenol.[6]

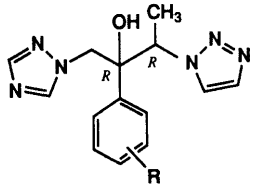
In the case of 1-triazolyl-2-butanol antifungals with the general formula I (Chart 1), which are known to have potent *in vivo* activity, the (2*R*,3*R*)-enantiomers such as Ia (SM-9164)[7] and Ib[8] were shown to have the most potent activity *in vivo* among the stereoisomers. However, there are few reports concerning the detailed relationships of the stereochemistry and the *in vivo* and *in vitro* antifungal activities among the four stereoisomers of these azolylbutanols (I). We previously reported the (2*R*,3*R*)-3-azolyl-2-(substituted phenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanols [(2*R*,3*R*)-**1a—d**, Chart 1] and their potent antifungal activities against *Candida albicans* (*C. albicans*) *in vivo*.[1] Thus, we planned to investigate the relationship between stereochemistry and antifungal activity using these azoles. In this paper, we describe the synthesis of the stereoisomers [(2*S*,3*S*)-, (2*R*,3*S*)-, (2*S*,3*R*)-**1a—d**] and clarify the relationship between the stereochemistry and the antifungal activity against *C. albicans in vitro* and *in vivo*.

On the other hand, although the interaction of azoles with cytochrome $P450_{14DM}$ in fungal cells is essential for their antifungal activity, a similar interaction of azoles with mammalian P450 has been reported to occur to some extent, and this could result in undesirable biological effects.[9] Therefore, a highly selective interaction with the



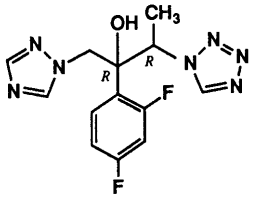
I

a: R = SO_2CH_3 (2*R*,3*R*)
b: R = SH (2*R*,3*R*)



(2*R*,3*R*)-**1a—c**

	R
a	2,4-F_2
b	2-F
c	4-F



(2*R*,3*R*)-**1d**

Chart 1

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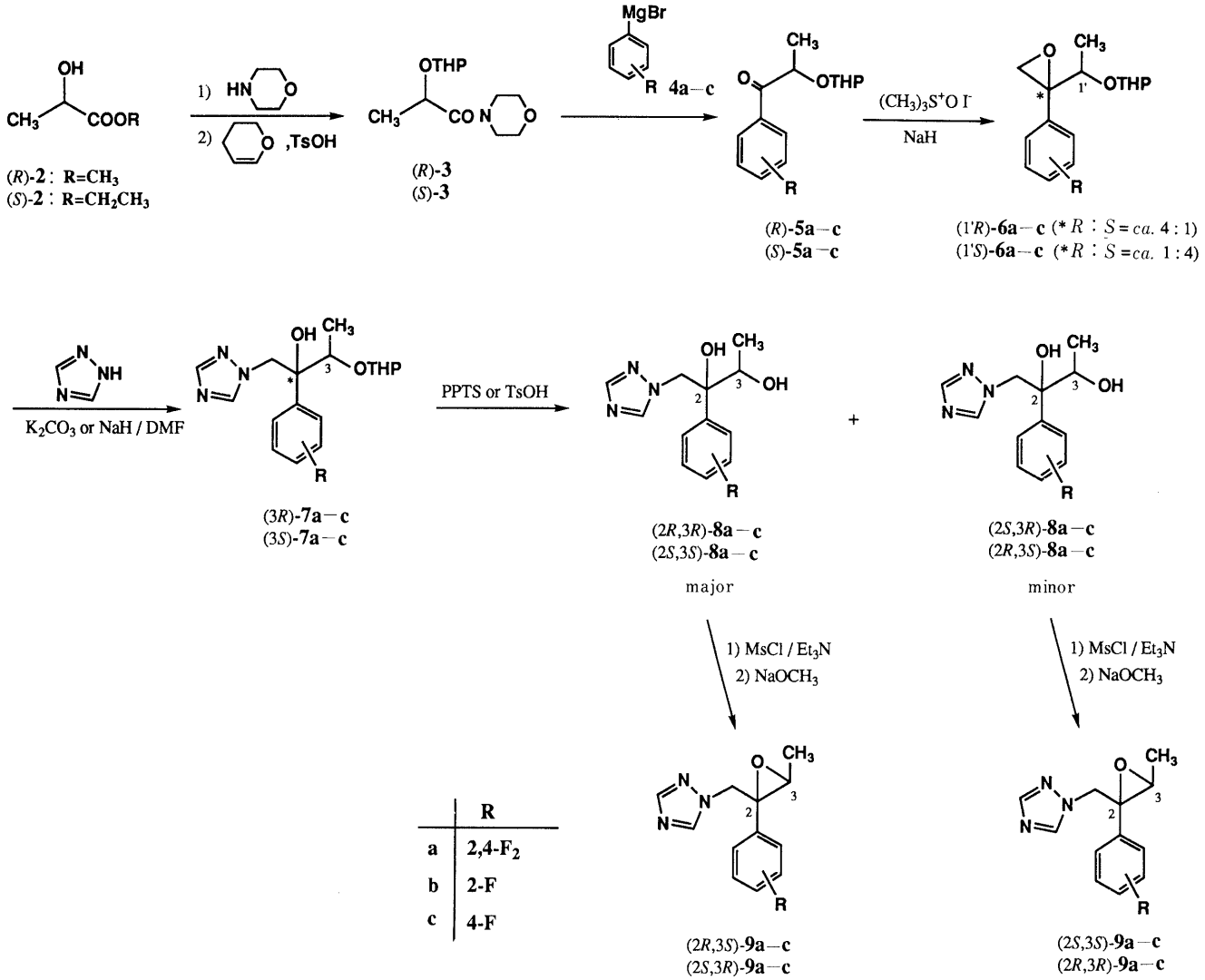


Chart 2

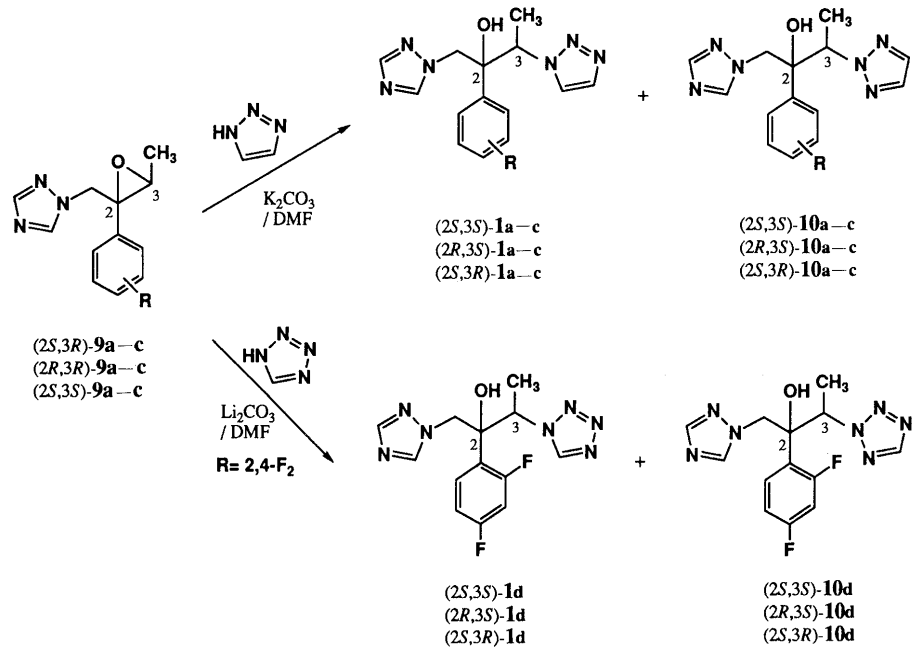
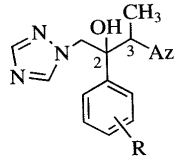


Chart 3

TABLE I. 3-Azolyl-2-(substituted phenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanols (**1a—d**)



No.	R	Az	Yield (%)	mp (°C) (Solvent)[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ (in $CDCl_3$)	IR ν (cm^{-1}) (KBr)	$[\alpha]_D$ (c, %) [°C] (in MeOH)	ee[b] [de][c] (%)
(2*R*,3*R*)-**1a**	2,4-F_2	1*H*-1,2,3-Triazol-1-yl[d]										>99[e] [>99.8]
(2*S*,3*S*)-**1a**	2,4-F_2	1*H*-1,2,3-Triazol-1-yl	35	118—119 (Et_2O)	$C_{14}H_{14}F_2N_6O$	52.50 (52.45)	4.41 (4.54)	26.24 (26.50)	1.38 (3H, d, J=7 Hz), 3.48 (1H, d, J=14.2 Hz), 4.98 (1H, d, J=14.2 Hz), 5.37 (1H, d, J=1.4 Hz), 5.52 (1H, q, J=7 Hz), 6.75—6.95 (2H, m), 7.42—7.68 (1H, m), 7.74 (1H, s), 7.76 (1H, s), 7.80 (1H, s), 7.98 (1H, s)	3128, 1618, 1596, 1500, 1419, 1274	+69.0 (1.0) {20}	>99.8[e] [>99.8]
(2*R*,3*S*)-**1a**	2,4-F_2	1*H*-1,2,3-Triazol-1-yl	44	140—155 (M–Et_2O)	$C_{14}H_{14}F_2N_6O$ ·2HCl	42.76 (42.96)	4.10 (4.32)	21.37 (21.45) [Cl, 18.03 (17.92)]	1.67 (3H, d, J=7 Hz), 4.84 (1H, d, J=14.8 Hz), 4.99 (1H, d, J=14.8 Hz), 5.49 (1H, q, J=7 Hz), 6.58—6.70 (1H, m), 6.88—7.10 (2H, m), 7.49 (1H, s), 7.90 (1H, s), 7.94 (1H, s), 8.73 (1H, s) (in DMSO-d_6)	3100, 3070, 1618, 1540, 1504, 1498	−29.7 (1.0) {20}	>99.8[f] [>99.7]
(2*S*,3*R*)-**1a**	2,4-F_2	1*H*-1,2,3-Triazol-1-yl	45	142—158 (M–Et_2O)	$C_{14}H_{14}F_2N_6O$ ·2HCl	42.76 (43.04)	4.10 (4.36)	21.37 (21.42) [Cl, 18.03 (17.89)]	1.66 (3H, d, J=7 Hz), 4.78 (1H, d, J=14.8 Hz), 4.98 (1H, d, J=14.8 Hz), 5.48 (1H, q, J=7 Hz), 6.58—6.70 (1H, m), 6.88—7.10 (2H, m), 7.48 (1H, s), 7.80 (1H, s), 7.94 (1H, s), 8.54 (1H, s) (in DMSO-d_6)	3108, 3074, 1618, 1558, 1540, 1500	+30.7 (1.0) {20}	>99.4[f] [>99.7]
(2*R*,3*R*)-**1b**	2-F	1*H*-1,2,3-Triazol-1-yl[d]										99[f] [>99.8]
(2*S*,3*S*)-**1b**	2-F	1*H*-1,2,3-Triazol-1-yl	44	118—119 (D–Et_2O)	$C_{14}H_{15}FN_6O$	55.62 (55.63)	5.00 (5.05)	27.80 (27.84)	1.38 (3H, d, J=7 Hz), 3.50 (1H, d, J=14.2 Hz), 5.03 (1H, d, J=14.2 Hz), 5.28 (1H, s), 5.59 (1H, q, J=7 Hz), 6.98—7.15 (2H, m), 7.18—7.36 (1H, m), 7.40—7.54 (1H, m), 7.73 (2H, s), 7.80 (1H, s), 8.00 (1H, s)	1610, 1580, 1513, 1483, 1450, 1274	+76.8 (1.0) {20}	>99[f] [>99.3]
(2*R*,3*S*)-**1b**	2-F	1*H*-1,2,3-Triazol-1-yl	43	124—129 (A–H)	$C_{14}H_{15}FN_6O$	55.62 (54.69)	5.00 (5.62)	27.80 (27.26)	1.78 (3H, d, J=7 Hz), 4.60 (1H, d, J=14 Hz), 5.09 (1H, d, J=14 Hz), 5.28 (1H, s), 5.42 (1H, q, J=7 Hz), 6.80—7.20 (4H, m), 7.49 (1H, s), 7.50 (1H, s), 7.76 (1H, s), 7.94 (1H, s)	1637, 1633, 1583, 1523, 1480, 1454	−8.9 (0.5) {20}	>99[f] [>99.3]
(2*S*,3*R*)-**1b**	2-F	1*H*-1,2,3-Triazol-1-yl	37	128—129 (A–H)	$C_{14}H_{15}FN_6O$	55.62 (52.22)	5.00 (5.13)	27.80 (27.55)	1.79 (3H, d, J=7 Hz), 4.61 (1H, d, J=14 Hz), 5.09 (1H, d, J=14 Hz), 5.29 (1H, s), 5.42 (1H, q, J=7 Hz), 6.78—7.20 (4H, m), 7.49 (1H, s), 7.50 (1H, s), 7.75 (1H, s), 7.94 (1H, s)	1637, 1633, 1583, 1523, 1486, 1444	+9.9 (0.5) {20}	>99.8[f] [>99.5]
(2*R*,3*R*)-**1c**	4-F	1*H*-1,2,3-Triazol-1-yl[d]										99.8[f] [>99.9]
(2*S*,3*S*)-**1c**	4-F	1*H*-1,2,3-Triazol-1-yl	39.5	155—157 (A)	$C_{14}H_{15}FN_6O$	55.62 (55.75)	5.00 (5.09)	27.80 (27.93)	1.40 (3H, d, J=7 Hz), 3.58 (1H, d, J=14.2 Hz), 4.60 (1H, d, J=14.2 Hz), 5.29 (1H, s), 5.30 (1H, q, J=7 Hz), 6.90—7.10 (2H, m), 7.25—7.40 (2H, m), 7.63 (1H, s), 7.79 (1H, s), 7.80 (1H, s), 7.98 (1H, s)	3260, 3126, 1604, 1540, 1510, 1274	+72.4 (1.0) {20}	>99.8[f] [>99.3]
(2*R*,3*S*)-**1c**	4-F	1*H*-1,2,3-Triazol-1-yl	29	Amorphous powder	$C_{14}H_{15}FN_6O$ ·HCl	49.64 (50.21)	4.76 (5.22)	24.81 (25.30)	1.68 (3H, d, J=7 Hz), 4.69 (1H, d, J=14.4 Hz), 4.96 (1H, d, J=14.4 Hz), 5.45 (1H, q, J=7 Hz), 6.82—6.98 (2H, m), 7.08—7.24 (2H, m), 7.51 (1H, s), 7.85 (1H, s), 7.92 (1H, br s), 8.34 (1H, br s) (in DMSO-d_6)	1606, 1558, 1540, 1508, 1456, 1228	−15.6 (1.0) {20}	99.4[e] [97]
(2*S*,3*R*)-**1c**	4-F	1*H*-1,2,3-Triazol-1-yl	27	Amorphous powder	$C_{14}H_{15}FN_6O$ ·HCl	49.64 (49.90)	4.76 (5.21)	24.81 (25.07)	1.63 (3H, d, J=7 Hz), 4.72 (1H, d, J=14.4 Hz), 4.98 (1H, d, J=14.4 Hz), 5.46 (1H, q, J=7 Hz), 6.84—7.00 (2H, m), 7.10—7.24 (2H, m), 7.52 (1H, s), 7.86 (1H, s), 8.00 (1H, br s), 8.49 (1H, br s) (in DMSO-d_6)	1606, 1558, 1540, 1508, 1456, 1228	+13.9 (1.0) {20}	96[e] [97]
(2*R*,3*R*)-**1d**	2,4-F_2	1*H*-1-Tetrazolyl[d]										>99.9[e] [>99.9]
(2*S*,3*S*)-**1d**	2,4-F_2	1*H*-1-Tetrazolyl	27	133—135 (E–H_2O)	$C_{13}H_{13}F_2N_7O$	48.60 (48.57)	4.08 (4.27)	30.52 (30.38)	1.42 (3H, d, J=7.2 Hz), 3.55 (1H, d, J=14 Hz), 4.98 (1H, d, J=14 Hz), 5.57 (1H, dq, J=1.6, 7.2 Hz), 5.63 (1H, s), 6.76—6.95 (2H, m), 7.37—7.55 (1H, m), 7.71 (1H, s), 7.78 (1H, s), 9.00 (1H, s)	3147, 1619, 1540, 1523, 1517, 1508	+46.4 (1.0) {20}	99.6[e] [>99.9]
(2*R*,3*S*)-**1d**	2,4-F_2	1*H*-1-Tetrazolyl	33	171—172 (A–Et_2O)	$C_{13}H_{13}F_2N_7O$	48.60 (48.62)	4.08 (4.17)	30.52 (30.57)	1.84 (3H, d, J=7.2 Hz), 4.57 (1H, d, J=14 Hz), 5.09 (1H, d, J=14 Hz), 5.44 (1H, d, J=7.2 Hz), 5.59 (1H, s), 6.52—6.78 (2H, m), 6.92—7.06 (1H, m), 7.81 (1H, s), 7.92 (1H, s), 8.72 (1H, s)	3122, 1621, 1600, 1540, 1519, 1504	+10.1 (1.0) {20}	99.1[f] [>99.9]
(2*S*,3*R*)-**1d**	2,4-F_2	1*H*-1-Tetrazolyl	30	171—172 (A–Et_2O)	$C_{13}H_{13}F_2N_7O$	48.60 (48.50)	4.08 (4.02)	30.52 (30.49)	1.84 (3H, d, J=7.2 Hz), 4.57 (1H, d, J=14 Hz), 5.09 (1H, d, J=14 Hz), 5.43 (1H, q, J=7.2 Hz), 5.58 (1H, s), 6.52—6.78 (2H, m), 6.92—7.06 (1H, m), 7.82 (1H, s), 7.92 (1H, s), 8.72 (1H, s)	3120, 1621, 1600, 1540, 1519, 1506	−12.0 (1.0) {20}	99.3[f] [>99.3]

a) Recrystallization solvents; D: dichloromethane, Et_2O: diethyl ether, M: methanol, IPE: diisopropyl ether, A: ethyl acetate, H: hexane. *b*) Enantiomer excess: determined by HPLC on a chiral column. *c*) Diasteromer excess: determined by HPLC on an ODS column. *d*) Ref. 1. *e*) Chiralcel OJ. *f*) Chiralcel OF.

fungal enzymes is one of the key factors determining the therapeutic utility of antifungal azoles. Hence, (2*R*,3*R*)-, (2*S*,3*S*)-, (2*R*,3*S*)- and (2*S*,3*R*)-**1a—d** were assayed for ergosterol biosynthesis-inhibitory activities using a cell lysate of *C. albicans* and for cholesterol biosynthesis-inhibitory activities using a cell lysate of rat liver in order to determine the relationships among selectivity, activity and configuration of the chiral centers in **1a—d**.

Chemistry

In the previous paper, we reported the synthesis of (2*R*,3*R*)-3-azolyl-2-(substituted phenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanols [(2*R*,3*R*)-**1a—d**] by the ring-opening reaction of (2*R*,3*S*)-3-methyl-2-(substituted phenyl)-2-(1*H*-1,2,4-triazol-1-yl)methyloxirane [(2*R*,3*S*)-**9a—c**] with 1*H*-1,2,3-triazole and 1*H*-tetrazole.[1] The optically active precursors [(2*R*,3*S*)-**9a—c**] were prepared from methyl (*R*)-lactate [(*R*)-**2**] *via* eight steps as shown in Chart 2. For the synthesis of stereoisomers [(2*S*,3*S*)-, (2*R*,3*S*)-, (2*S*,3*R*)-**1a—d**], the corresponding oxiranes [(2*S*,3*R*)-, (2*R*,3*R*)-, (2*S*,3*S*)-**9a—c**] were required as precursors. Among them, the (2*S*,3*R*)- oxiranes [(2*S*,3*R*)-**9a—c**] were synthesized using ethyl (*S*)-lactate [(*S*)-**2**] as the starting material and following a route similar to that used for the synthesis of (2*R*,3*S*)-**9a—c**, as shown in Chart 2.[10] On the other hand, the diastereomeric isomers [(2*S*,3*S*)-, (2*R*,3*R*)-**9a—c**] were prepared from the minor components of the diastereomeric mixtures which were formed at the step of epoxidation (**5a—c**→**6a—c**). The diols (2*S*,3*R*)- and (2*R*,3*S*)-**8a—c** were separated by silica gel chromatography and converted to (2*S*,3*S*)- and (2*R*,3*R*)-**9a—c**, respectively.[10]

The oxiranes [(2*S*,3*R*)-, (2*R*,3*R*)- and (2*S*,3*S*)-**9a—c**] were reacted with 1*H*-1,2,3-triazole in the presence of K_2CO_3 or 1*H*-tetrazole in the presence of Li_2CO_3 to give (2*S*,3*S*)-, (2*R*,3*S*)- and (2*S*,3*R*)-**1a—d**, respectively, in 27—45% yields (Chart 3, Table I). The regio-isomeric by-products **10a—d** of this reaction were removed by silica gel chromatography.

For the accurate comparison of biological activities, it was considered to be essential to assess the diastereomeric purity (% de) and the enantiomeric purity (% ee) of each isomer. The % de and % ee were determined by HPLC on an octadecyl silica (ODS) column and a chiral stationary phase column, respectively. The results are shown in Table I. Most of the compounds had high diastereomeric purity (>99% de) as well as high optical purity (>99% ee) except for (2*R*,3*S*)- and (2*S*,3*R*)-**1c**, which were not crystallized and were obtained as amorphous powders.

Biological Activity

The four sets of optically active azoles [(2*R*,3*R*)-, (2*S*,3*S*)-, (2*R*,3*S*)-, (2*S*,3*R*)-**1a—d**] were evaluated for *in vitro* antifungal activity against two strains of *C. albicans*, TA and IFO 0583. The *in vitro* assay was carried out by a paper disc method (Disc)[8] using yeast nitrogen base medium (YNB), by an agar-dilution method[11] using YNB and by a microbroth dilution method using synthetic amino acid medium, fungal (SAAMF).[12] The *in vitro* activities are expressed as the diameter (mm) of the growth inhibition zone around the paper disc soaked in a 1 mg/ml solution of the test compound and as the minimum inhibitory concentration (MIC; μg/ml). In addition, the *in vitro* activity of azoles to inhibit the hyphal outgrowth of *C. albicans* IFO 0583 in serum[13] was measured and expressed in terms of the MIC against the hyphal outgrowth (MICH; μg/ml).[14]

C. albicans TA-infected mice were used for the *in vivo* assay,[8] and the activity is expressed in terms of ED_{50} (mg/kg; the oral dose of the test compound which allowed 50% of the infected mice to survive). The results of these

TABLE II. Biological Activity of 3-Azolyl-2-(substituted phenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanols (**1a—d**)

Compound	Antifungal activity against *C. albicans* *In vitro*						*In vivo*	Sterol biosynthesis-inhibitory activity IC_{50} (μg/ml)	
	Disc (mm)		MIC (μg/ml)			MICH[a] (μg/ml)	ED_{50} po (mg/kg)		
	IFO 0583 YNB	TA YNB	IFO 0583 YNB	TA YNB	TA SAAMF	IFO 0583	TA	*C. albicans* TA	Rat liver
(2*R*,3*R*)-**1a**	50	45	>100	>100	1.56	0.1	0.28	0.0019	270
(2*S*,3*S*)-**1a**	17	14	>100	>100	>100	>20	>16[b]	>1	>1000[c]
(2*R*,3*S*)-**1a**	39	37	100	100	12.5	0.62	3.4	0.012	>1000[c]
(2*S*,3*R*)-**1a**	17	18	>100	>100	>100	>20	>16	>1	>1000
(2*R*,3*R*)-**1b**	47	18	>100	>100	1.56	0.05	0.35	0.0026	260
(2*S*,3*S*)-**1b**	27	25	100	>100	25	3.2	6.2	0.25	>1000[c]
(2*R*,3*S*)-**1b**	34	30	100	>100	12.5	0.8	7.1	0.030	>1000
(2*S*,3*R*)-**1b**	21	17	>100	>100	>100	50	>16	>1	>1000
(2*R*,3*R*)-**1c**	35	36	>100	>100	6.25	0.4	0.45	0.0057	260
(2*S*,3*S*)-**1c**	30	28	100	100	100	3.2	>16[b]	0.21	760
(2*R*,3*S*)-**1c**	35	36	50	50	25	1.6	10	0.019	610
(2*S*,3*R*)-**1c**	14	14	100	100	>100	>20	>16	0.70	>1000
(2*R*,3*R*)-**1d**	38	40	>100	>100	3.13	0.4	0.38	0.0025	610
(2*S*,3*S*)-**1d**	13	12	>100	>100	>100	>20	>16	0.62	790
(2*R*,3*S*)-**1d**	25	20	100	100	100	10	14.3	0.027	>1000[c]
(2*S*,3*R*)-**1d**	0	0	>100	>100	>100	>20	>16	>1	>1000[c]
Fluconazole	42	18	>100	>100	3.13	0.2—0.8	0.29—0.35	0.0027	>1000

a) MIC against hyphal growth. *b*) The compound postponed the death of mice at 16 mg/kg. *c*) Partially inhibited at 1000 μg/ml.

assays are shown in Table II.

All the stereoisomers of the azoles (**1a—d**) except (2*S*,3*R*)-**1d** inhibited the growth of *C. albicans* IFO 0583 and TA in the paper disc assay. The results indicated that (2*R*,3*R*)-isomers have the strongest activity, (2*R*,3*S*)-isomers are the next most potent and (2*S*,3*S*)- and (2*S*,3*R*)-isomers are slightly weaker. The susceptibilities of IFO 0583 and TA to these azoles were similar in this assay.

The observed MIC values for *C. albicans* IFO 0583 and TA on YNB were 50—100 μg/ml or more, within the range often observed with triazole antifungals, and no clear difference in the values was seen among the stereoisomers. On the other hand, the MIC values on SAAMF and the MICH values were clearly dependent on the configuration of the azole compounds. The (2*R*,3*R*)-isomers showed the lowest MICs (1.56—6.25 μg/ml) on SAAMF and the strongest inhibitory activity (MICH; 0.05—0.4 μg/ml) against hyphal outgrowth. The order of potency was (2*R*,3*R*)$\gg$(2*R*,3*S*)$>$(2*S*,3*S*)$\geq$(2*S*,3*R*). This order is the same as that observed in the paper disc assay, but the activity differences among the stereoisomers are more clear in the MIC on SAAMF and the MICH assays.

In the *in vivo* assay, activities were also found to be dependent on configuration. The potency paralleled the *in vitro* MIC (SAAMF) and the MICH values, and the order was (2*R*,3*R*)$\gg$(2*R*,3*S*)$\geq$(2*S*,3*S*)$\geq$(2*S*,3*R*). Among the four stereoisomers of each set, the (2*R*,3*R*)-isomer had a protective effect comparable to that of fluconazole against *C. albicans* TA infection in mice. The ED_{50} values of the (2*R*,3*R*)-isomers were 0.28 mg/kg [(2*R*,3*R*)-**1a**], 0.35 mg/kg [(2*R*,3*R*)-**1b**], 0.45 mg/kg [(2*R*,3*R*)-**1c**] and 0.38 mg/kg [(2*R*,3*R*)-**1d**],[1] indicating potency 12—37 times that of the corresponding (2*R*,3*S*)-isomer. From the clear relationship between the activities *in vitro* and *in vivo*, the differences in the *in vivo* activity among the stereoisomers are considered to be due to differences in the intrinsic activity of the azoles and not in the pharmacokinetics or oral absorption.

The stereoisomers [(2*R*,3*R*)-, (2*S*,3*S*)-, (2*R*,3*S*)- and (2*S*,3*R*)-**1a—d**] were assessed for inhibitory activity *in vitro* against sterol 14α-demethylase of *C. albicans* TA and rat liver using the cell lysate and [^{14}C]mevalonic acid. The activities are expressed in terms of IC_{50} (μg/ml: the concentration of azole required to give 50% inhibition of incorporation of radioactivity into desmethylated sterols), and the results are shown in Table II.

A clear difference in inhibitory activity among the stereoisomers of **1a—d** was observed in the sterol biosynthesis of *C. albicans* TA. Differences in fungal cell membrane permeability should be negligible, because a cell lysate was used for the assay. As shown in Table II, the order of the inhibitory potency in *C. albicans* TA was (2*R*,3*R*)$\gg$(2*R*,3*S*)$>$(2*S*,3*S*)$\geq$(2*S*,3*R*), which parallels the antifungal activities *in vitro* and *in vivo*. The (2*R*,3*R*)-isomers caused potent inhibition (IC_{50}: 0.0019—0.0057 μg/ml). These results indicate that the (2*R*,3*R*)-configuration of **1a—d** provides the best fit to cytochrome $P450_{14DM}$ of *C. albicans*. On the other hand, the effects of **1a—d** on the sterol synthesis in rat liver were weak (IC_{50}: 260$\rightarrow>$1000 μg/ml) with relatively small differences among the stereoisomers. Thus, the (2*R*,3*R*)-isomers of **1a—d** proved to be the most potent and selective inhibitors of cytochrome $P450_{14DM}$ of *C. albicans*.

As described above, the *in vivo* antifungal activities of the stereoisomers clearly paralleled the *in vitro* activities: MIC (SAAMF), MICH and IC_{50} (sterol biosynthesis). Therefore, it is concluded that the target enzyme, cytochrome $P450_{14DM}$, of *C. albicans* clearly recognizes the configuration of the two chiral centers in the azoles (**1a—d**), preferring especially the (*R*)-configuration at the C2 position, and this is reflected in the *in vivo* antifungal potency.

Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with a Horiba FT-200 Fourier-transform IR spectrometer. ^{1}H-NMR spectra were taken on a Varian Gemini-200 spectrometer with tetramethylsilane as the internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. The optical rotations were recorded with a JASCO DIP-370 digital polarimeter. The secondary ion mass spectra (SIMS) were obtained on a Hitachi M-80A mass spectrometer.

Reactions were followed by TLC on TLC plates, Silica gel 60 F_{254} precoated (E. Merck). Chromatographic separations were carried out on Silica gel 60 (0.063—0.200 mm, E. Merck).

The % de of **1a—d** was determined by HPLC using an ODS column (A-303; 4.6 mm i.d. × 250 mm, Yamamura Chemical Laboratories Co.) under the following conditions: mobile phase, MeOH–H_2O–acetic acid, 7 : 3 : 0.02, v/v; flow rate 0.8 ml/min; detection, UV at 262 nm.

The % ee of **1a—d** was determined by HPLC using a chiral stationary phase column (Chiralcel OJ and OF, 4.6 mm i.d. × 250 mm, Daicel Chemical Industries, Tokyo, Japan) under the following conditions [column, mobile phase, flow rate, detection]: (2*R*,3*R*)-**1a**, (2*S*,3*S*)-**1a**, (2*R*,3*R*)-**1d** and (2*S*,3*S*)-**1d** [OJ, hexane–EtOH (7 : 3), 1 ml/min, UV at 262 nm]; (2*R*,3*S*)-**1a** and (2*S*,3*R*)-**1a** [OF, hexane–EtOH (7 : 3), 1 ml/min, UV at 262 nm]; (2*R*,3*R*)-**1b**, (2*S*,3*S*)-**1b**, (2*R*,3*S*)-**1b** and (2*S*,3*R*)-**1b** [OF, hexane–isopropyl alcohol (7 : 3), 1 ml/min, UV at 262 nm] ; (2*R*,3*R*)-**1c**, (2*S*,3*S*)-**1c**, (2*R*,3*S*)-**1c**, (2*S*,3*R*)-**1c**, (2*R*,3*S*)-**1d** and (2*S*,3*R*)-**1d** [OF, hexane–isopropyl alcohol (1 : 1), 1 ml/min, UV at 262 nm].

(2*S*)-4′-Fluoro-2-(3,4,5,6-tetrahydro-2*H*-pyran-2-yloxy)propiophenone [(*S*)-5c] A mixture of Mg (turnings, 5.98 g, 246 mmol) and **4c** (20 g, 114 mmol) in tetrahydrofuman (THF) (200 ml) was stirred vigorously to initiate the reaction. When the reaction temperature reached 40 °C, the mixture was cooled to 35 °C in an ice bath and **4c** (23 g, 132 mmol) was added dropwise to the mixture over a period of 5 min, keeping the reaction temperature at 35—40 °C. The mixture was stirred at room temperature for 1 h and then cooled at 0 °C in an ice bath. A solution of (*S*)-**3** (50 g, 205 mmol)[8] in THF (60 ml) was added dropwise to the mixture over a period of 13 min in an argon atmosphere. The resulting mixture was stirred at room temperature for 2 h, then a saturated aqueous solution of ammonium chloride (aqueous NH_4Cl, 100 ml) and water (100 ml) were added, and the whole was extracted with ethyl acetate (AcOEt, 500 ml). The extract was washed successively with water (100 ml × 2) and brine (100 ml) and dried over magnesium sulfate ($MgSO_4$). The solvent was evaporated *in vacuo* and the residue was chromatographed on silica gel (500 g). Elution with hexane–AcOEt (30 : 1$\rightarrow$5 : 1, v/v) gave (*S*)-**5c** (51.4 g, quantitative) as a pale yellow oil. IR (neat): 2942, 1697, 1598, 1506 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.46, 1.53 (3H, d each, J = 7 Hz), 1.40—1.92 (6H, m), 3.29—3.59 (1H, m), 3.61—3.98 (1H, m), 4.57—4.80 (1H, m), 4.92, 5.18 (1H, q each, J = 7 Hz), 7.06—7.18 (2H, m), 8.03—8.17 (2H, m).

(2*S*)-2′-Fluoro-2-(3,4,5,6-tetrahydro-2*H*-pyran-2-yloxy)propiophenone [(*S*)-5b] Mg (turnings, 4.4 g, 181 mmol) was added to a mixture of **4b** (15 g, 85.7 mmol) and (*S*)-**3** (40 g, 164 mmol) in THF (200 ml) and the resulting mixture was stirred vigorously to initiate the reaction. When the reaction temperature reached 35 °C, the mixture was cooled in a water bath and **4b** (16.7 g, 95.3 mmol) was added dropwise to the mixture over a period of 10 min, keeping the reaction temperature at 35—37 °C. The resulting mixture was stirred at 30—35 °C for 2 h then cooled in an ice bath. Aqueous NH_4Cl (100 ml) and water (100 ml) were added, and

the whole was extracted with AcOEt (200 ml × 2, 100 ml). The extracts were combined, washed successively with water (100 ml) and brine (50 ml), and then dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was chromatographed on silica gel (400 g). Elution with hexane–AcOEt (10 : 1→5:1, v/v) gave (*S*)-**5b** (22.4 g, 55%) as a pale yellow oil. IR (neat): 2942, 1697, 1608, 1558, 1540, 1456 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.40—2.00 (9H, m), 3.29—3.60 (1H, m), 3.65—3.98 (1H, m), 4.65—4.88 (1H, m), 4.92, 5.15 (1H, q each, J=7 Hz), 7.08—7.29 (2H, m), 7.43—7.60 (1H, m), 7.78—7.90 (1H, m).

2-(4-Fluorophenyl)-2-[(1*S*)-1-(3,4,5,6-tetrahydro-2*H*-pyran-2-yloxy)-ethyl]oxirane [(1′*S*)-6c] Under a nitrogen atmosphere, trimethylsulfoxonium iodide (54 g, 245 mmol) was added portionwise to a stirred mixture of NaH (60% oil dispersion, 9.4 g, 235 mmol) and dimethylsulfoxide (DMSO) (350 ml) under ice cooling over a period of 40 min. The resulting mixture was stirred at room temperature for 30 min and then cooled in an ice bath. A solution of (*S*)-**5c** (51.4 g, 203 mmol) in DMSO (60 ml) was added and the whole was stirred at room temperature for 2 h. The mixture was poured into cold water (500 ml) and extracted with AcOEt (400 ml, 200 ml × 2). The extracts were combined, washed successively with water (100 ml × 3) and brine (100 ml), and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give (1′*S*)-**6c** (55.5 g) as a pale yellow oil, which contained mineral oil and was used for the next step without purification.

In a similar manner, (1′*S*)-**6b** (21.4 g) was prepared from (*S*)-**5b** (21.1 g) and used for the next step without purification.

(3*S*)-2-(4-Fluorophenyl)-3-(3,4,5,6-tetrahydro-2*H*-pyran-2-yloxy)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol [(3*S*)-7c] A mixture of (1′*S*)-**6c** obtained above (27.8 g), 1*H*-1,2,4-triazole (13.4 g, 193 mmol), K_2CO_3 (40.2 g, 291 mmol) and DMF (300 ml) was stirred at 80 °C for 16 h. After being cooled, the mixture was concentrated *in vacuo* to *ca.* 100 ml. The residue was diluted with AcOEt (500 ml) and the insoluble material was filtered off. The filtrate was washed successively with water (100 ml × 2) and brine (100 ml) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (300 g). Elution with hexane–AcOEt (1 : 1)→AcOEt–acetone (4 : 1) gave (3*S*)-**7c** [27.2 g, 79% based on (*S*)-**5c**] as a white solid mass.

^{1}H-NMR ($CDCl_3$) δ: 0.99, 1.13 (3H, d each, J=6.4 Hz), 1.40—1.95 (6H, m), 3.40—3.60 (1H, m), 3.74—4.17 (2H, m), 4.30—5.01 (4H, m), 6.90—7.55 (4H, m), 7.74—8.07 (2H, m).

The reaction of (1′*S*)-**6b** obtained above (21.4 g) with 1*H*-1,2,4-triazole was carried out in a manner similar to that described above to give (3*S*)-**7b** [22 g, 78.5% based on (*S*)-**5b**]. ^{1}H-NMR ($CDCl_3$) δ: 0.99, 1.12 (3H, d each, J=6.2 Hz), 1.40—2.00 (6H, m), 3.45—3.65 (1H, m), 3.80—4.28 (2H, m), 4.29—5.00 (4H, m), 6.90—7.12 (2H, m), 7.14—7.32 (1H, m), 7.40—7.52 (1H, m), 7.71—8.10 (2H, m).

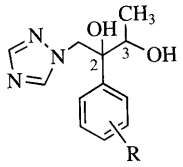
(2*S*,3*S*)-2-(4-Fluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2,3-butanediol [(2*S*,3*S*)-8c, Table III] and (2*R*,3*S*)-2-(4-Fluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2,3-butanediol [(2*R*,3*S*)-8c, Table III] TsOH hydrate (15.4 g, 81.1 mmol) was added to a solution of (3*S*)-**7c** (27.2 g, 81.1 mmol) in MeOH (200 ml). The mixture was stirred at room temperature for 1 h and then neutralized with a 2 N NaOH aqueous solution (40.5 ml). The resulting mixture was concentrated *in vacuo*, and the residue was extracted with AcOEt (500 ml). The extract was washed successively with water (100 ml) and brine (50 ml), and dried over $MgSO_4$. Evaporation of the solvent and crystallization of the residue from a mixture of diethyl ether (Et_2O) and diisopropyl ether (iso-Pr_2O) (3 : 1, v/v, 40 ml) gave (2*S*,3*S*)-**8c** (10.4 g, 51%) as colorless prisms. The mother liquor was concentrated *in vacuo* and the residue was chromatographed on silica gel (350 g, AcOEt–MeOH, 30 : 1, v/v). The eluent containing the less polar isomer was concentrated to give (2*R*,3*S*)-**8c** (3 g) as an oil. This oil was treated with 4 N HCl in AcOEt and crystallized from MeOH–AcOEt to afford (2*R*,3*S*)-**8c** · HCl (3.02 g, 13%) as a colorless crystalline powder. The eluent containing the more polar isomer was concentrated to give additional (2*S*,3*S*)-**8c** (4.8 g, 23.6%).

The diols (2*S*,3*S*)- and (2*R*,3*S*)-**8b** (Table III) were prepared from (3*S*)-**7b** in a manner similar to that described above.

The diols (2*S*,3*R*)-**8b**—**c** (Table III) were obtained from the mother liquors after the separation of (2*R*,3*R*)-**8b**—**c**.[1]

(2*S*,3*R*)-2-(4-Fluorophenyl)-3-methyl-2-(1*H*-1,2,4-triazol-1-yl)methyloxirane [(2*S*,3*R*)-9c, Table IV] Methanesulfonyl chloride (7.23 g,

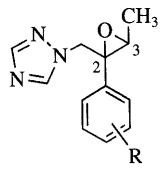
TABLE III. 2-(Substituted phenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2,3-butanediols (**8**)



No.	R	Yield (%)	mp (°C) (Solvent)[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR (in $CDCl_3$) δ	IR ν (KBr) cm^{-1}	$[\alpha]_D$ (c, %) {°C} (in MeOH)
(2*R*,3*R*)-**8b**	2-F[b]									
(2*S*,3*S*)-**8b**	2-F	61.6	65—66 (Et_2O)	$C_{12}H_{14}FN_3O_2$	57.36 (57.30)	5.62 (5.58)	16.72 (16.80)	0.98 (3H, d, J=6.4 Hz), 2.70 (1H, d, J=9.2 Hz), 4.30—4.50 (1H, m), 4.72 (1H, s), 4.80 (1H, d, J=14.2 Hz), 4.90 (1H, d, J=14.2 Hz), 6.94—7.32 (3H, m), 7.38—7.50 (1H, m), 7.81 (1H, s), 7.82 (1H, s)	3400, 1518, 1489, 1446, 1270, 1211	+84.9 (0.51) {23}
(2*R*,3*S*)-**8b**	2-F	9.3	Oil	$C_{12}H_{14}FN_3O_2$	SIMS: 252 (MH^+)			1.27 (3H, d, J=6.6 Hz), 2.53 (1H, d, J=5.4 Hz), 3.98—4.10 (1H, m), 4.58 (1H, d, J=14.4 Hz), 4.93 (1H, s), 5.08 (1H, d, J=14.4 Hz), 6.90—7.30 (3H, m), 7.50—7.62 (1H, m), 7.77 (1H, s), 8.03 (1H, s)	3370, 1612, 1580, 1510, 1450, 1275	−68.6 (1.0) {20}
(2*S*,3*R*)-**8b**	2-F	6.1	Oil	$C_{12}H_{14}FN_3O_2$	SIMS: 252 (MH^+)			1.28 (3H, d, J=6.6 Hz), 2.53 (1H, br), 3.98—4.10 (1H, m), 4.58 (1H, d, J=14.4 Hz), 4.94 (1H, s), 5.09 (1H, d, J=14.4 Hz), 6.90—7.30 (3H, m), 7.50—7.63 (1H, m), 7.78 (1H, s), 8.04 (1H, s)	3370, 1612, 1580, 1510, 1450, 1275	+64.6 [0.62] (22)
(2*R*,3*R*)-**8c**	4-F[b]									
(2*S*,3*S*)-**8c**	4-F	74.6	99—101 (A–H)	$C_{12}H_{14}FN_3O_2$	57.36 (57.48	5.62 5.68	16.72 16.66)	0.97 (3H, d, J=6.4 Hz), 2.76 (1H, br), 4.02—4.22 (1H, m), 4.31 (1H, s), 4.54 (1H, d, J=14 Hz), 4.72 (1H, d, J=14 Hz), 6.90—7.05 (2H, m), 7.14—7.28 (2H, m), 7.72 (1H, s), 7.88 (1H, s)	3421, 1604, 1516, 1508, 1411, 1280	+60.7 (1.0) {20}
(2*R*,3*S*)-**8c**	4-F	13	145—165 (M–A)	$C_{12}H_{14}FN_3O_2$ · HCl	50.09 (49.91	5.25 5.25	14.72 14.72)	0.82 (3H, d, J=6.2 Hz), 3.74 (1H, q, J=6.2 Hz), 4.67 (1H, d, J=14.5 Hz), 4.79 (1H, d, J=14.5 Hz), 7.00—7.20 (2H, m), 7.45—7.60 (2H, m), 8.17 (1H, br s), 8.73 (1H, br s) (in DMSO-d_6–D_2O)	3350, 3100, 1560, 1537, 1506, 1427	−71.0 (0.92) {20}
(2*S*,3*R*)-**8c**	4-F	7.4	140—162 (M–A)	$C_{12}H_{14}FN_3O_2$ · HCl	50.09 (50.12	5.25 5.22	14.72 14.77)	0.84 (3H, d, J=6.2 Hz), 3.78 (1H, q, J=6.2 Hz), 4.65 (1H, d, J=14.5 Hz), 4.79 (1H, d, J=14.5 Hz), 7.00—7.18 (2H, m), 7.45—7.60 (2H, m), 8.17 (1H, br s), 8.73 (1H, br s) (in DMSO-d_6–D_2O)	3350, 3100, 1562, 1537, 1506, 1427	+69.1 (1.0) {20}

a) Recrystallization solvents: A, ethyl acetate; H, hexane; M, methanol; Et_2O, diethyl ether. *b*) Ref. 1.

TABLE IV. 3-Methyl-2-(substituted phenyl)-2-(1*H*-1,2,4-triazol-1-yl)methyloxiranes (**9**)



No.	R	Yield (%)	mp (°C) (Solvent)[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR (in $CDCl_3$) δ	IR ν (KBr) cm^{-1}	$[\alpha]_D$ (c, %) {°C} (in MeOH)
(2*R*,3*S*)-**9b**	2-F[b]									
(2*S*,3*R*)-**9b**	2-F	72	74—76 (IPE)	$C_{12}H_{12}FN_3O$	61.79 (61.87	5.19 5.17	18.02 18.02)	1.65 (3H, d, J=5.6 Hz), 3.22 (1H, q, J=5.6 Hz), 4.46 (1H, d, J=14.6 Hz), 4.92 (1H, d, J=14.6 Hz), 6.94—7.10 (3H, m), 7.18—7.31 (1H, m), 7.81 (1H, s), 7.92 (1H, s)	1543, 1508, 1489, 1448, 1275, 1213	+7.3 (1.0) {20}
(2*R*,3*R*)-**9b**	2-F	84	Oil	$C_{12}H_{12}FN_3O$	61.79 (61.53	5.19 5.27	18.02 18.41)	1.06 (3H, d, J=5.4 Hz), 3.16 (1H, d, J=5.4 Hz), 4.47 (1H, d, J=14.8 Hz), 4.82 (1H, d, J=14.8 Hz), 7.00—7.40 (4H, m), 7.85 (1H, s), 8.05 (1H, s)	1506, 1490, 1456, 1273, (film)	−5.5 (1.06) {20}
(2*S*,3*S*)-**9b**	2-F	81	Oil	$C_{12}H_{12}FN_3O$	61.72 (61.52	5.19 5.22	18.02 18.39)	1.06 (3H, d, J=5.4 Hz), 3.16 (1H, d, J=5.4 Hz), 4.47 (1H, d, J=14.8 Hz), 4.82 (1H, d, J=14.8 Hz), 7.00—7.40 (4H, m), 7.85 (1H, s), 8.05 (1H, s)	1508, 1492, 1456, 1275, (film)	+6.0 (1.0) {20}
(2*R*,3*S*)-**9c**	4-F[b]									
(2*S*,3*R*)-**9c**	4-F	92	52—53 (IPE)	$C_{12}H_{12}FN_3O$	61.79 (61.65	5.19 5.21	18.02 17.90)	1.64 (3H, d, J=5.6 Hz), 3.17 (1H, q, J=5.6 Hz), 4.45 (1H, d, J=14.6 Hz), 4.85 (1H, d, J=14.6 Hz), 6.90—7.08 (2H, m), 7.08—7.25 (2H, m), 7.88 (1H, s), 7.94 (1H, s)	3114, 1604, 1508, 1272, 1220, 1134	+9.5 (1.0) {20}
(2*R*,3*R*)-**9c**	4-F	76	52—53 (IPE–H)	$C_{12}H_{12}FN_3O$	61.79 (61.90	5.19 5.02	18.02 18.09)	1.00 (3H, d, J=5.4 Hz), 3.06 (1H, q, J=5.4 Hz), 4.54 (1H, d, J=14.8 Hz), 4.66 (1H, d, J=14.8 Hz), 6.95—7.14 (2H, m), 7.14—7.30 (2H, m), 7.90 (1H, s), 8.03 (1H, s)	3100, 3002, 1607, 1515, 1508, 1456	−6.2 (1.06) {20}
(2*S*,3*S*)-**9c**	4-F	84	52—53 (IPE–H)	$C_{12}H_{12}FN_3O$	61.79 (62.02	5.19 5.16	18.02 18.00)	1.00 (3H, d, J=5.4 Hz), 3.06 (1H, q, J=5.4 Hz), 4.54 (1H, d, J=14.8 Hz), 4.66 (1H, d, J=14.8 Hz), 6.95—7.14 (2H, m), 7.14—7.30 (2H, m), 7.91 (1H, s), 8.03 (1H, s)	3100, 3002, 1608, 1515, 1508, 1456	+6.4 (1.0) {20}

a) Recrystallization solvents: IPE, diisopropyl ether; H, hexane. *b*) Ref. 1.

63.1 mmol) was added to a mixture of (2*S*,3*S*)-**8c** (13.2 g, 52.5 mmol), triethylamine (8.79 ml, 63.1 mmol), AcOEt (50 ml) and CH_2Cl_2 (200 ml) at 0 °C. The mixture was stirred at 0 °C for 1 h, then washed successively with water (100 ml) and brine (50 ml), and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* gave an oil, which was dissolved in a mixture of NaOMe (28% in MeOH, 11.1 g, 57.8 mmol) and MeOH (200 ml). The mixture was stirred at room temperature for 15 min and then concentrated *in vacuo*. The residue was extracted with AcOEt (150 ml), and the extract was washed successively with water (50 ml) and aqueous solution of sodium bicarbonate (aqueous $NaHCO_3$), and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was crystallized from iso-Pr_2O (30 ml) to give (2*S*,3*R*)-**9c** (9.02 g, 73%) as colorless needles. The mother liquor was concentrated and purified by silica gel column chromatography (70 g). Elution with AcOEt–CH_2Cl_2 (4 : 1, v/v) followed by crystallization from a mixture of iso-Pr_2O and hexane gave additional (2*S*,3*R*)-**9c** (2.29 g). (2*R*,3*R*)- and (2*S*,3*S*)-**9c** (Table VI) were prepared from (2*R*,3*S*)- and (2*S*,3*R*)-**8c** in a manner similar to that described above.

The 2-fluoro analogues (2*S*,3*R*)-, (2*R*,3*R*)- and (2*S*,3*S*)-**9b** (Table IV) were obtained by a method similar to that employed for the synthesis of **9c**.

(2*S*,3*S*)-2-(4-Fluorophenyl)-3-(1*H*-1,2,3-triazol-1-yl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol [(2*S*,3*S*)-1c, Table I] and (2*S*,3*S*)-2-(4-Fluorophenyl)-3-(2*H*-1,2,3-triazol-2-yl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol [(2*S*,3*S*)-10c, Table V] A mixture of (2*S*,3*R*)-**9c** (2.0 g, 8.58 mmol), 1*H*-1,2,3-triazole (1.18 g, 17.1 mmol) and K_2CO_3 (3.56 g, 25.7 mmol) in DMF (40 ml) was heated at 80 °C for 20 h. The mixture was cooled, then AcOEt (100 ml) was added and the insoluble material was filtered off. The filtrate was evaporated *in vacuo* and the residue was dissolved in AcOEt (200 ml). The organic layer was washed with brine (100 ml) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was chromatographed on silica gel (70 g). Elution with AcOEt–CH_2Cl_2 (4 : 1, v/v) followed by crystallization from CH_2Cl_2–iso-Pr_2O gave (2*S*,3*S*)-**10c** (1.15 g) as a less polar substance. Elution with CH_2Cl_2–acetone (1 : 1, v/v) followed by crystallization from AcOEt gave (2*S*,3*S*)-**1c** (0.98 g) as a colorless crystalline powder. (2*R*,3*S*)- and (2*S*,3*R*)-**1c** (Table I) were prepared in a manner similar to that described above.

The reaction of (2*S*,3*R*)-**9a—b**, (2*R*,3*R*)-**9a—b** and (2*S*,3*S*)-**9a—b** with 1*H*-1,2,3-triazole in the presence of K_2CO_3 was carried out in a manner similar to that described above to give 1*H*-1,2,3-triazol-1-yl [(2*S*,3*S*)-**1a—b**, (2*R*,3*S*)-**1a—b** and (2*S*,3*R*)-**1a—b**, Table I] and 2*H*-1,2,3-triazol-2-yl [(2*S*,3*S*)-**10a—b**, (2*R*,3*S*)-**10a—b** and (2*S*,3*R*)-**10a—b**, Table V] derivatives.

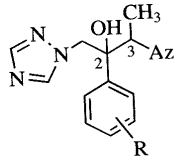
(2*S*,3*S*)-2-(2,4-Difluorophenyl)-3-(1*H*-tetrazol-1-yl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol [(2*S*,3*S*)-1d, Table I] and (2*S*,3*S*)-2-(2,4-Difluorophenyl)-3-(2*H*-tetrazol-2-yl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol [(2*S*,3*S*)-10d, Table V] A mixture of (2*S*,3*R*)-**9a** (0.30 g, 1.19 mmol), 1*H*-tetrazole (0.167 g, 2.39 mmol), Li_2CO_3 (0.88 g, 11.9 mmol) and DMF (6 ml) was heated at 110 °C for 22 h. After being cooled, the mixture was diluted with AcOEt (50 ml), washed successively with water (10 ml) and brine (10 ml), and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was chromatographed on silica gel (40 g, AcOEt–CH_2Cl_2, 4 : 1, v/v). The eluent containing the less polar isomer was concentrated to give (2*S*,3*S*)-**10d** (0.136 g) as an amorphous powder. This powder was treated with HCl in AcOEt and crystallized from Et_2O to give (2*S*,3*S*)-**10d** · HCl (0.15 g) as a colorless powder. The eluent containing the more polar isomer was concentrated. The residue was crystallized from EtOH–water to give (2*S*,3*S*)-**1d** (0.104 g) as colorless needles.

The reaction of (2*R*,3*R*)-, (2*S*,3*S*)-**9a** with 1*H*-tetrazole in the presence of Li_2CO_3 was carried out in a manner similar to that described above to give 1-tetrazolyl [(2*R*,3*S*)-, (2*S*,3*R*)-**1d**, Table I] and 2-tetrazolyl [(2*R*,3*S*)-, (2*S*,3*R*)-**10d**, Table V] derivatives.

***In Vitro* MICH Assay** *C. albicans* IFO 0583 cells were grown overnight at 28 °C in peptone-yeast extract-glucose medium, then washed with water, suspended in bovine serum at 2×10^6 cells/ml, and incubated in a microtiter plate at 37 °C for 18 h in the presence of azoles. The MICH (μg/ml) was determined under a microscope and defined as the lowest concentration which inhibits the hyphal outgrowth of cells.

Sterol Biosynthesis Assay The method of Barrett-Bee *et al.*[15] was modified for the preparation of a cell lysate of *C. albicans*. *C. albicans* TA cells were collected from a late exponential phase culture, suspended

TABLE V. 3-(2-Azolyl)-2-(substituted phenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanols (**10**)



No.	R	Az	Yield (%)	mp (°C) (Solvent)[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ (in $CDCl_3$)	IR ν (cm^{-1}) (KBr)	$[\alpha]_D$ (c, %) [°C] (in MeOH)
(2*R*,3*R*)-**10a**	2,4-F_2	2*H*-1,2,3-Triazol-2-yl[b]									
(2*S*,3*S*)-**10a**	2,4-F_2	2*H*-1,2,3-Triazol-2-yl	28	99—100 (IPE)	$C_{14}H_{14}F_2N_6O$	52.50 (52.64	4.41 4.59	26.24 26.47)	1.43 (3H, d, J=7 Hz), 3.57 (1H, d, J=14.4 Hz), 4.90 (1H, d, J=14.4 Hz), 5.28 (1H, s), 5.53 (1H, q, J=7 Hz), 6.75—6.92 (2H, m), 7.43—7.63 (1H, m), 7.65 (1H, s), 7.77 (2H, s), 7.85 (1H, s)	3334, 1616, 1558, 1540, 1506, 1498	+83.7 (1.0) {20}
(2*R*,3*S*)-**10a**	2,4-F_2	2*H*-1,2,3-Triazol-2-yl	45	116—128 (A–Et_2O)	$C_{14}H_{14}F_2N_6O$ ·HCl	47.13 (46.89	4.26 4.60	23.56 23.58)	1.65 (3H, d, J=7 Hz), 4.76 (1H, d, J=14.4 Hz), 4.96 (1H, d, J=14.4 Hz), 5.41 (1H, q, J=7 Hz), 6.68—6.80 (1H, m), 6.95—7.10 (2H, m), 7.60 (2H, s), 7.79 (1H, s), 8.45 (1H, br s) (in DMSO-d_6)	3274, 1618, 1598, 1558, 1540, 1506	−41.2 (1.0) {20}
(2*S*,3*R*)-**10a**	2,4-F_2	2*H*-1,2,3-Triazol-2-yl	46	115—129 (A–Et_2O)	$C_{14}H_{14}F_2N_6O$ ·HCl	47.13 (46.82	4.24 4.44	23.56 23.44)	1.64 (3H, d, J=7 Hz), 4.72 (1H, d, J=14.4 Hz), 4.96 (1H, d, J=14.4 Hz), 5.40 (1H, q, J=7 Hz), 6.62—6.76 (1H, m), 6.88—7.10 (2H, m), 7.55 (2H, s), 7.77 (1H, s), 8.43 (1H, br s) (in DMSO-d_6)	3274, 1616, 1598, 1558, 1540, 1506	+39.8 (1.0) {20}
(2*R*,3*R*)-**10b**	2-F	2*H*-1,2,3-Triazol-2-yl[b]									
(2*S*,3*S*)-**10b**	2-F	2*H*-1,2,3-Triazol-2-yl	30	151—165 (E–Et_2O)	$C_{14}H_{15}FN_6O$ ·HCl	49.64 (49.80	4.76 4.79	24.81 24.91)	1.37 (3H, d, J=7 Hz), 3.92 (1H, d, J=14.4 Hz), 4.94 (1H, d, J=14.4 Hz), 5.42 (1H, q, J=7 Hz), 7.00—7.40 (4H, m), 7.78 (1H, s), 7.92 (2H, s), 8.51 (1H, s) (in DMSO-d_6)	3370, 1620, 1590, 1550, 1525, 1488	+83.2 (0.5) {20}
(2*R*,3*S*)-**10b**	2-F	2*H*-1,2,3-Triazol-2-yl	49	113—121 (M–Et_2O)	$C_{14}H_{15}FN_6O$ ·HCl	49.64 (49.02	4.76 5.01	24.81 24.34)	1.78 (3H, d, J=7 Hz), 4.60 (1H, d, J=14 Hz), 5.09 (1H, d, J=14 Hz), 5.28 (1H, s), 5.42 (1H, q, J=7 Hz), 6.80—7.20 (4H, m), 7.49 (1H, s), 7.50 (1H, s), 7.76 (1H, s), 7.94 (1H, s) (in DMSO-d_6)	3400, 1614, 1583, 1562, 1537, 1486	−35.9 (0.5) {20}
(2*S*,3*R*)-**10b**	2-F	2*H*-1,2,3-Triazol-2-yl	46	120—124 (M–Et_2O)	$C_{14}H_{15}FN_6O$ ·HCl	49.64 (49.31	4.76 5.00	24.81 24.66)	1.79 (3H, d, J=7 Hz), 4.61 (1H, d, J=14 Hz), 5.09 (1H, d, J=14 Hz), 5.29 (1H, s), 5.42 (1H, q, J=7 Hz), 6.78—7.20 (4H, m), 7.49 (1H, s), 7.50 (1H, s), 7.75 (1H, s), 7.94 (1H, s) (in DMSO-d_6)	3450, 1614, 1583, 1562, 1537, 1486	+36.3 (0.5) {20}
(2*R*,3*R*)-**10c**	4-F	2*H*-1,2,3-Triazol-2-yl[b]									
(2*S*,3*S*)-**10c**	4-F	2*H*-1,2,3-Triazol-2-yl	50.2	125—127 (D–IPE)	$C_{14}H_{15}FN_6O$	55.62 (55.41	5.00 4.98	27.80 27.47)	1.43 (3H, d, J=7 Hz), 3.73 (1H, d, J=14.2 Hz), 4.55 (1H, d, J=14.2 Hz), 5.22 (1H, s), 5.28 (1H, q, J=7 Hz), 6.90—7.10 (2H, m), 7.20—7.40 (2H, m), 7.71 (1H, s), 7.75 (3H, s)	3421, 1604, 1510, 1456, 1417, 1338	+90.6 (1.0) {20}
(2*R*,3*S*)-**10c**	4-F	2*H*-1,2,3-Triazol-2-yl	57	92—94 (A–IPE)	$C_{14}H_{15}FN_6O$	55.62 (55.57	5.00 5.10	27.80 27.69)	1.78 (3H, d, J=6.8 Hz), 4.46 (1H, d, J=14.2 Hz), 5.67 (1H, d, J=14.2 Hz), 5.39 (1H, q, J=6.8 Hz), 5.41 (1H, s), 6.75—6.90 (2H, m), 6.90—7.10 (2H, m), 7.45 (2H, s), 7.76 (1H, s), 7.88 (1H, br s)	3480, 3128, 1608, 1519, 1508, 1421	−8.6 (1.0) {20}
(2*S*,3*R*)-**10c**	4-F	2*H*-1,2,3-Triazol-2-yl	53	92—94 (A–IPE)	$C_{14}H_{15}FN_6O$	55.62 (55.45	5.00 5.09	27.80 27.75)	1.78 (3H, d, J=6.8 Hz), 4.46 (1H, d, J=14.2 Hz), 4.67 (1H, d, J=14.2 Hz), 5.39 (1H, q, J=6.8 Hz), 5.41 (1H, s), 6.75—6.90 (2H, m), 6.90—7.08 (2H, m), 7.45 (2H, s), 7.75 (1H, s), 7.88 (1H, s)	3480, 3128, 1608, 1519, 1508, 1421	+8.3 (1.0) {20}
(2*R*,3*R*)-**10d**	2,4-F_2	2*H*-2-Tetrazolyl[b]									
(2*S*,3*S*)-**10d**	2,4-F_2	2*H*-2-Tetrazolyl	36	127—139 (Et_2O)	$C_{13}H_{13}F_2N_7O$ ·HCl	43.65 (43.42	3.94 4.02	27.41 27.35)	1.49 (3H, d, J=7 Hz), 4.18 (1H, d, J=14.4 Hz), 4.92 (1H, d, J=14.4 Hz), 5.67 (1H, q, J=7 Hz), 6.90—7.04 (1H, m), 7.20—7.40 (2H, m), 7.71 (1H, s), 8.31 (1H, s), 9.10 (1H, s) (in DMSO-d_6)	3336, 3066, 1618, 1560, 1540, 1504	+51.0 (1.0) {20}
(2*R*,3*S*)-**10d**	2,4-F_2	2*H*-2-Tetrazolyl	50	120—123 (Et_2O)	$C_{13}H_{13}F_2N_7O$	48.60, (47.98	4.08 4.22	30.52 30.17)	1.81 (3H, d, J=7 Hz), 4.70 (1H, d, J=14 Hz), 5.02 (1H, dd, J=14, 1.2 Hz), 5.32 (1H, s), 5.60 (1H, q, J=7 Hz), 6.58—6.72 (2H, m), 7.18—7.32 (1H, m), 7.78 (1H, s), 8.03 (1H, s), 8.39 (1H, s)	3133, 1619, 1596, 1511, 1504, 1457	−11.5 (1.0) {20}
(2*S*,3*R*)-**10d**	2,4-F_2	2*H*-2-Tetrazolyl	51	120—123 (Et_2O)	$C_{13}H_{13}F_2N_7O$	48.60 (48.01	4.08 4.30	30.52 30.02)	1.81 (3H, d, J=7 Hz), 4.69 (1H, d, J=14 Hz), 5.02 (1H, dd, J=14, 1.2 Hz), 5.32 (1H, s), 5.60 (1H, q, J=7 Hz), 6.58—6.72 (2H, m), 7.18—7.32 (1H, m), 7.78 (1H, s), 8.03 (1H, s), 8.39 (1H, s)	3133, 1619, 1596, 1511, 1502, 1457	+10.0 (1.0) {20}

a) Recrystallization solvents; D: dichloromethane, Et_2O: diethyl ether, M: methanol, IPE: diisopropyl ether, A: ethyl acetate, H: hexane. *b*) Ref. 1.

at 2×10^9 cells/ml in 100 mM potassium phosphate buffer (pH 7.4) containing 1 mM EDTA, 2 mM glutathione, 4 mM $MgCl_2$ and 20% glycerol, and disrupted with glass beads (diameter, 0.25—0.45 mm) in a Bead-Beater homogenizer (Bio Spec, U.S.A.) for a total of 12.5 min (0.5 min pulses with 2 min intervals) in an ice-water bath. A cell lysate of rat liver was prepared according to the method of Moir *et al.*[16] with some modifications. Female F344/Du rat (Charles River, Japan) were fed with powder diet containing 3% cholestylamine. The liver was added to 100 mM potassium phosphate buffer (pH 7.4) containing 1 mM EDTA, 2 mM glutathione, 4 mM $MgCl_2$ and 20% glycerol (three times the volume of the liver) and homogenized for 2 min with an Ultra-Turrax homogenizer. The homogenates of *C. albicans* and rat liver were centrifuged at 5000 and 10000 ***g***, respectively, for 20 min at 4 °C and the supernatant, of which the protein content was adjusted to 4 mg/ml, was used for the assay.

Inhibitory activity of azoles against sterol biosynthesis in the cell lysate prepared above was measured according to the method of Barrett-Bee *et al.*[15] with slight modifications. The reaction mixture (0.5 ml), which consisted of 1.8 mg protein of the lysate, 0.25 μCi (40 mCi/mmol) of [^{14}C]mevalonic acid (Amersham, Japan), the test compound, 1% DMSO and an NADPH generating system (0.5 μmol of NADPH, 0.5 μmol of NADP, 0.5 μmol of NAD, 1.5 mmol of glucose-6-phosphate, 2. μmmol of reduced glutathione, 0.35 i.u. of glucose-6-phosphate dehydrogenase, 1 μmol of $MnCl_2$ and 1.5 μmol of $MgCl_2$), was incubated at 37 °C for 60 min. The reaction was stopped by adding 1 ml of 2.7 M KOH in 90% (v/v) EtOH, and the resulting mixture was kept at 70 °C for 60 min. The non-saponifiable lipids were extracted with petroleum ether and submitted to TLC on a Kieselgel 60 F_{254} plate (toluene–Et_2O, 9 : 1, v/v). The TLC plate was then exposed to a Kodak X-Omat film. The incorporation of radioactivity into desmethylated sterols was measured by densitometry of the autoradiogram, and inhibitory activity of the azoles against sterol biosynthesis was expressed in terms of IC_{50} (the concentration of azoles giving 50% decrease in the density of the desmethylated fraction).

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