condensation product 12 was debenzoylated to give the ribofuranoside 13.

Determination of the Amount of MH-Ribofuranoside (13) To Be Used in This Study. In order to determine the dosage to be used in our incorporation studies, a test was conducted involving the injection of various amounts (0.0–8.5 mg, in increments of 0.5 mg) of 13 dissolved in propylene glycol (total volume 0.5 mL) into fertilized chick eggs. Since the injection of the propylene glycol itself (Table I) resulted, on average, in a 40% death rate (presumably due to defects in technique), a 60% survival rate with the 13 dissolved in it was considered the best level possible. Consequently, a dosage of 3.0 mg in propylene glycol (0.5 mL) was used for our incorporation studies.

Incorporation Studies of 13 in Fertilized Chick Eggs. The genetic material from the test eggs and the controls, which had been purified and separated into fractions, was counted for the presence of labeled MH-ribofuranoside in a Packard Tri-Carb 4530 scintillation counter. When none of the ^{14}C seemed to be present in any of the test material (Table II), the droppings of chicks that had been allowed to come to term and hatch and that had been isolated since birth were counted. The total weight of 3 days of droppings was about 40 g, indicating a total count of about 180 000 or about a 66.5% recovery of injected radioactivity.

Conclusions. It is clear that, as is the case with MH itself, MH-ribofuranoside (13) is not incorporated into the animal cells of developing chick embryos. Thus, it may not be able to be used as a therapeutic treatment for localized tumors. All of the radioactivity of the injected nucleoside was found in the excretion of the hatched eggs. As the embryo develops, it feeds off the yolk and this continues for several days after hatching. Since chicks have only one mode of excretion, we cannot say whether the nucleoside was broken down in the gastrointestinal tract and the MH absorbed and, with or without biotransformation, eliminated (as with the rats) or whether it was passed unchanged into the cloaca.

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Structural Studies of the Vinyltriazole Fungicide Diniconazole (ER Pure) Related to Its Binding to Cytochrome P-450

Toshiyuki Katagi

The conformation of diniconazole (ER pure) [(*R*)-(*E*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol, Ia] in a lower energy state was optimized by semiempirical molecular orbital calculations. The optimized conformer was supported by the presence of a hydrogen bond between the hydroxy proton and the nitrogen atom at the 2-position of the 1,2,4-triazolyl moiety, as measured with infrared and nuclear magnetic resonance spectrometers. The substrate difference spectra with the rat liver microsomal enzymes indicated that a racemic compound of Ia was stoichiometrically bound to cytochrome P-450 enzymes. It was strongly suggested that Ia binds to cytochrome P-450 enzymes via the N4 lone pair of the 1,2,4-triazolyl moiety. The primary mode of action of Ia in fungi was discussed by the computer-aided superimposition of the optimized conformation of Ia on that of lanosterol, which was the intermediate in the ergosterol biosynthesis pathways.

Diniconazole (ER pure) [(*R*)-(*E*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol, Ia] is a systemic fungicide showing fungitoxicity to a broad range of fungal species (Funaki et al., 1984). Among the possible four isomers, Ia shows the highest fungicidal activity (Sasaki, 1985). As with other fungicides possessing the triazolyl or imidazolyl group in the molecule (Gadher et al., 1983), Ia is known to inhibit the biosynthesis of ergosterol in fungal species (Takano et al., 1983).

The following studies have been performed to elucidate the mode of action of these fungicides: (1) GC–MS analysis of the intermediates of ergosterol biosynthesis (Aoyama and Yoshida, 1978); (2) substrate difference spectra of microsomal enzymes prepared from rat liver or yeast cells in the presence of a fungicide (Henry and Sisler, 1984); (3) computer graphics to visualize a structural similarity between a fungicide and lanosterol derivatives (Sugavanam, 1984; Marchington, 1983). The results of these studies strongly suggest that the primary mode of action of fun-

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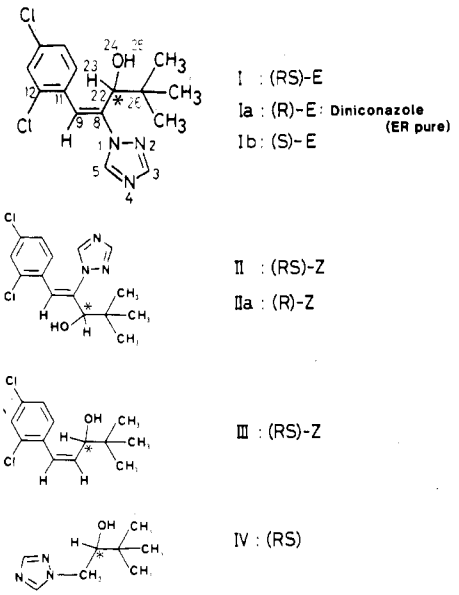

Figure 1. Chemical structures and designations of Ia and its related compounds.

gicides is the competitive inhibition of cytochrome P-450 enzymes (Gibbons et al., 1979), which catalyze the oxidation of the C14 methyl group of lanosterol. Henry and Sisler (1984) demonstrated that the function of the sterol carrier proteins or enzyme-modulating phospholipids was little affected. As X-ray crystallographic data of the enzyme active site are not available except those of cytochrome $P\text{-}450_{cam}$ (Poulos et al., 1985), the study of the molecular shape and the quantum chemical properties of a substrate have been discussed in relation to the proposed enzyme site (Marchington, 1983). The structural study of azolylmethanes to discuss the dominant conformations in solution (Anderson et al., 1984) might suit this strategy.

This report deals with the conformations of Ia and its related compounds in solution, together with the quantum chemical calculations and the binding assay with the microsomal enzymes, to elucidate the mode of action in a molecular level.

MATERIALS AND METHODS

Chemicals. The chemical structures of Ia and its related compounds are shown in Figure 1. The racemic compound I and the corresponding *Z* isomer II were synthesized in our laboratory (Funaki et al., 1984). The most biologically active (Ia) and the corresponding *S-E* isomer (Ib) were separated from I by high-performance liquid chromatography. A Hitachi 638-50 liquid chromatograph equipped with a Sumipax OA-4200 column (5 μm, 4-mm i.d. × 25-cm length; Sumika Analysis Service Ltd., Osaka) was used for separation. The mobile phase was hexane–1,2-dichloroethane–ethanol (700:70:5) and eluted at a flow rate of 0.8 mL/min. The eluate from the column was monitored at 250 nm with a Hitachi 635M UV detector. The retention times of Ia and Ib were 41.2 and 38.0 min, respectively. The optical purities of Ia and Ib were >99 %.

(*E*)-1-(2,4-Dichlorophenyl)-4,4-dimethyl-1-penten-3-one was synthesized by aldol condensation (Nielsen et al., 1968) of 2,4-dichlorobenzaldehyde (Aldrich Chemical Co., Milwaukee) with *tert*-butyl methyl ketone (Tokyo Kasei Co. Ltd., Tokyo) and subsequent photoisomerization to the *Z* isomer in acetonitrile–acetone (3:1) by UV light from a 400-W high-pressure mercury vapor lamp (Taika Kogyo, Osaka). The resulting compound was reduced with sodium borohydride in methanol to obtain III.

α-Bromomethyl *tert*-butyl ketone was prepared by treatment of *tert*-butyl methyl ketone with bromine in carbon tetrachloride (CCl_4). The brominated derivative was reacted with 1*H*-1,2,4-triazole (Aldrich) in acetonitrile in the presence of potassium carbonate and then reduced with sodium borohydride to obtain IV. The products were purified by a silica gel (Kieselgel 60, 70–230 mesh; E. Merck, Darmstadt) column chromatography and precoated silica gel chromatoplates (Kieselgel $60F_{254}$, 20 × 20 cm, 0.25-mm layer thickness; Merck).

Spectroscopy. Proton nuclear magnetic resonance (^1H NMR) spectra were measured with a Hitachi R-90H Fourier transform (FT) spectrometer at 90 MHz in chloroform-*d* ($CDCl_3$). The concentration of Ia or Ib was adjusted to 0.06 or 0.006 mol/L. Each sample solution was thermostated by circulating the nitrogen gas cooled by liquid nitrogen to the desired temperature (+25, 0, −25, −50 °C). The temperature fluctuated within 1 °C during the measurements. FT infrared (IR) spectra were measured in CCl_4 with a Digilab FTS-20E FT-IR spectrometer by using a potassium bromide cell (2-mm path length) at 25 °C. The concentration was varied from 0.002 to 0.1 mol/L. The substrate difference spectrum was measured with a Hitachi 557DB spectrophotometer by using a quartz cuvette (1-cm path length).

Preparation of Rat Liver Microsomal Enzymes and Binding Assay. Rat liver microsomes were prepared from male Sprague–Dawley rats (200 g). A 20% (w/v) homogenate of rat livers in 1.15% potassium chloride was centrifuged at 9000*g* for 20 min to obtain the supernatant fraction, which was in turn centrifuged at 105000*g* for 60 min to sediment microsomes. The microsomal pellet was washed by resuspension and centrifugation in phosphate buffer (pH 7.4). The content of cytochrome P-450 in suspension was spectrophotometrically determined by the method of Omura and Sato (1964). Six milliliters of microsomal suspension was divided into two curvettes, and after the base correction the spectral change caused by the addition of I or II in methanol was recorded. The binding constant of fungicide (K_s) was estimated according to the method reported by Schenkman et al. (1967).

Theoretical Studies. Semiempirical molecular orbital calculations were carried out by using a MNDO program (QCPE No. 353) (Dewar and Thiel, 1977) loaded on a IBM 370-158MVS computer. The initial geometry used for calculations was derived as follows. The molecular geometries of (2,4-dichlorophenyl)ethylene and 1*H*-1,2,4-triazole were separately optimized by an energy gradient method of the MNDO program. Then, the molecular geometries of Ia, Ib, and IIa were derived, supposing that the alkyl C–C and C–H bond lengths were 1.52 and 1.10 Å, respectively, and that the bond angles of alkyl C–C–C and C–C–H were 109.5°. The dihedral angles were initially defined to realize the staggered conformation. Finally, the six dihedral angles of Ia and Ib, defined as shown in Table I, were optimized by calculations. In the case of *R-Z* isomer (IIa), the steric hindrance between the two rings was found to mislead the optimization of geometry. Therefore, two dihedral angles defining the conformation of the phenyl and triazolyl rings were estimated by measurement of the spin–lattice relaxation time (T_1) (Katagi, 1985). The T_1 value of a proton is known to be dependent on the neighboring atoms (Farrar and Becker, 1971). The proton and carbon atoms at the 6-position of the phenyl ring and those at the 5-position of the triazolyl ring were used as a probe to study the geometry of the *Z* isomer. By comparison of the observed T_1 with the calculated T_1 of the speculated configuration, two dihedral angles of the phenyl

Table I. Results of MNDO Calculations for Ia and Ib

dihedral angle, deg	Ia conformer			Ib conformer		
	1	2		1	2	
N2–N1–C8–C9	51.423	132.538		301.835	48.095	
C8–C9–C11–C12	283.077	246.588		236.849	274.884	
C9–C8–C22–H23	15.665	211.464		336.520	142.625	
C9–C8–C22–O24	125.978	326.429		219.090	25.659	
C8–C22–O24–H25	95.000	210.875		77.083	115.847	
C9–C8–C22–C26	260.461	95.153		94.705	260.776	
ΔE^a	0.000	3.788		0.000	3.309	
charge^b	−0.2712	−0.2725		−0.2731	−0.2718	
$E_L{}^c$	−11.7646	−11.7044	−11.8126	−11.6401	−11.6740	−11.7843
$(S_D)^d$	(1.036)	(0.5576)	(0.4288)	(0.9802)	(0.4828)	(0.1642)

[a] Difference of total energy relative to conformer 1 (kcal/mol). [b] The partial charge of the N4 atom of the 1,2,4-triazolyl moiety. [c] The energy level of the N4 lone pair (eV). [d] The electron density of the N4 lone pair.

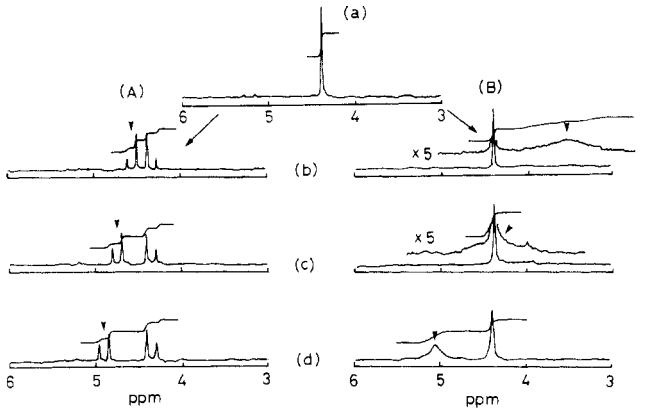

Figure 2. ^{1}H NMR spectra of Ia in $CDCl_3$: (A) 0.006 mol/L; (B) 0.06 mol/L (a) +25 °C, (b) 0 °C, (c) −25 °C, (d) −50 °C. The arrows indicate the hydroxy proton signal. (A) The coupling constant of the doublet is 10 Hz. (B) The chemical shift (ppm) values of the hydroxy proton are 3.56 (b), 4.40 (c), or 5.07 (d).

and triazolyl rings to the olefinic C=C double bond were estimated to be 210.0 and 30.0°, respectively.

The computer graphics were displayed by using a ACACS system (Yoshida et al., 1985), developed by Sumitomo Chemical Co., Ltd., and Nippon Electric Co. Ltd., loaded on a NEC ACOS System 430 computer. The contour map of electron density of a molecular orbital was calculated and displayed by using the obtained eigenvectors and Slater-type atomic orbitals. The superimposition was also conducted by a ACACS system to visualize the similarlity of the molecular shapes between two chemicals. The three-dimensional structure of 24,25-dibromolanost-8-en-3-β-yl acetate was referred to the Cambridge X-ray crystallographic data base (Carlisle and Timmins, 1974) and used for the superimposition.

RESULTS

Structural Studies in Solution. The ^{1}H NMR spectra of Ia at several temperatures are shown in Figure 2. The singlet peak at 4.40 ppm having an intensity of two protons, appearing in (a), was assigned to *CHOH* because the addition of deuterium oxide reduced the intensity by one proton. The larger temperature dependence of chemical shift of the hydroxy proton was observed at 0.06 mol/L (ca. −0.03 ppm/deg) than at 0.006 mol/L (ca. −0.006 ppm/deg). At 0.06 mol/L, the hydroxy proton showed a broad signal due to a rapid exchange of the proton with other one. In contrast, the doublet peak caused by a coupling with the methine proton was clearly observed at 0.006 mol/L. These results strongly suggest that the hydroxy proton has an intermolecular hydrogen bond at a higher concentration and a weak intramolecular hydrogen bond at a lower concentration.

The presence of the hydrogen bonds was ascertained by comparison of the FT-IR spectra of Ia and Ib with those of the synthetic analogues III and IV, as shown in Figure 3. In the case of III, which did not possess the 1,2,4-triazolyl moiety in the molecule, a typical absorption spectrum due to the free hydroxy group was observed at 3260 cm^{-1} at concentrations of up to 0.1 mol/L. This indicated that little hydrogen bond was formed between the hydroxy proton and the π-electrons of the phenyl ring. In contrast, IV exhibited two broad absorptions, together with one sharp absorption due to the free hydroxy group. On the basis of the concentration dependence and the

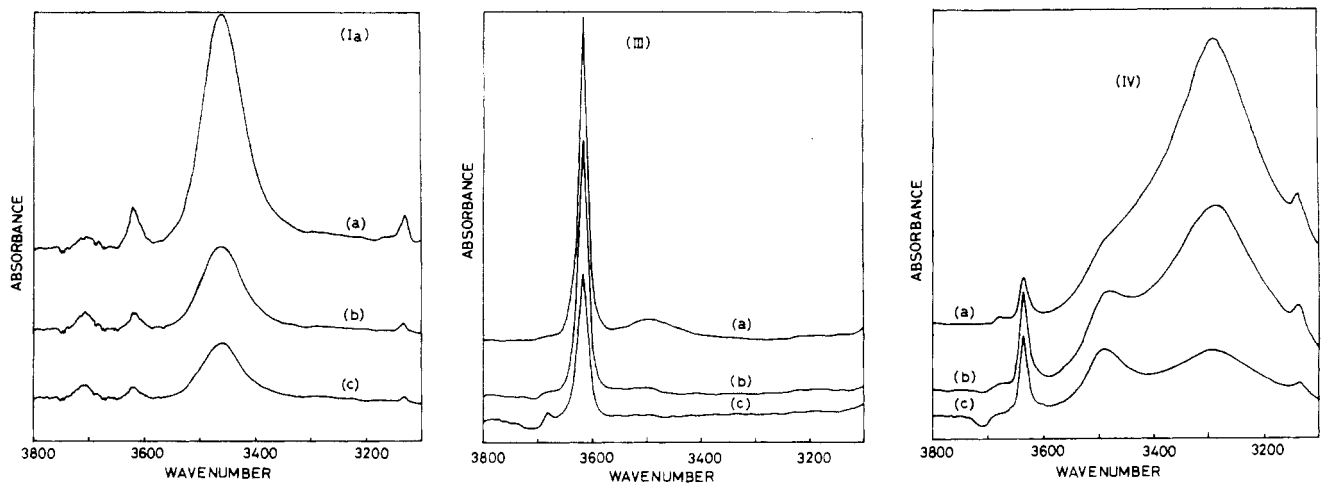

Figure 3. FT-IR spectra: (Ia) (a) 0.01, (b) 0.004, (c) 0.002 mol/L; (III and IV) (a) 0.1, (b) 0.02, (c) 0.005 mol/L in CCl_4. The absorption maxima (cm^{-1}) are 3461 (Ia), 3620 (III), and 3640, 3490, and 3280 (IV).

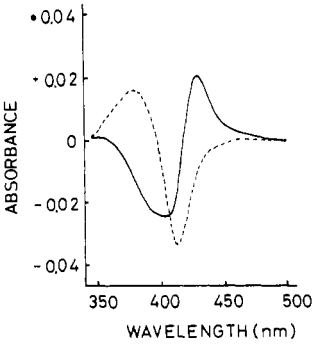

Figure 4. Difference spectra of rat liver microsomal enzymes (2.6 μmol/L) caused by the addition of I (3 μmol/L) or II (50 μmol/L): (I) solid line, absorption peak at 426 nm and a trough at 392 nm; (II) dotted line, absorption peak at 380 nm and a trough at 412 nm.

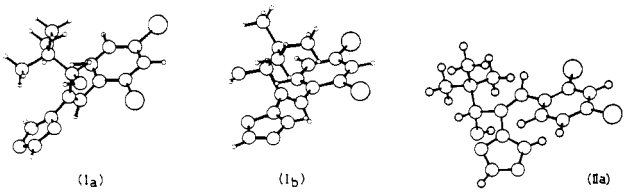

Figure 5. Absolute configuration of Ia, Ib, (conformer 1), and IIa optimized by MNDO calculations.

characteristic wavenumber, the absorptions at 3280 and 3490 cm^{-1} of IV seem to be due to the intermolecular and intramolecular hydrogen bonds, respectively (Nakanishi, 1960). Supposing that the dominant conformations of Ia, III, and IV are similarly realized in CCl_4, the broad absorption of Ia at 3461 cm^{-1} is assigned to the intramolecular hydrogen bond. Ia and Ib were spectroscopically identical.

Binding Assay. The typical substrate difference spectra of microsomal enzymes in the presence of I or II are shown in Figure 4. The spectral change of the microsomal enzymes caused by I was a type II spectrum (Schenkman et al., 1967). The difference of absorbance ($A_{426} - A_{392}$) was proportional to the increase of fungicide concentration of up to ca. 1.5 μmol/L and was saturated at above ca. 2.5 μmol/L. By the reciprocal plot of ($A_{426} - A_{392}$) and the concentration of the fungicide, the binding constant, K_s, was estimated to be 4.2 μmol/L. These results indicate the stoichiometric binding of I to cytochrome P-450. In contrast, II caused the so-called type I difference spectrum. As compared with I, about a 10-fold amount of II was required to cause the same extent of the spectral change. These results indicated that II was bound to cytochrome P-450 enzyme in a different way from I.

Conformations of Ia and Ib. The ball-and-stick models of Ia and Ib (conformer 1) are shown in Figure 5, together with that of IIa, which is obtained by MNDO calculation and T_1 measurements. The optimized dihedral angles, the relative difference of total energy, and the physicochemical properties of the N4 lone pair of the 1,2,4-triazolyl moiety are listed in Table I. There was no significant difference in the energy level and the electron density of the N4 lone pair IIa, as compared with Ia and Ib. The conformational change from conformer 1 to conformer 2 resulted in the splitting of the molecular orbital of the N4 lone pair, leading to the diffusion of the lone-pair electron density. Conformer 1 of Ia was about 3–4 kcal/mol more stable than conformer 2. For the dominant comformer 1 of Ia, the contour map of the N4 lone pair is shown in Figure 6.

Computer Graphics. As conformer 1 of Ia in a lower energy state possessed the higher electron density at the

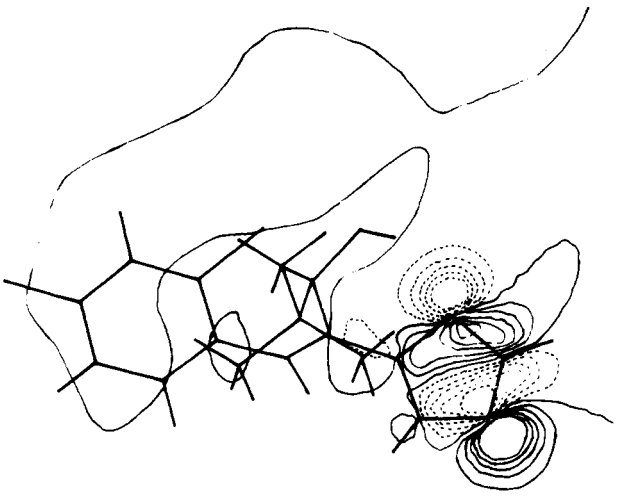

Figure 6. Contour map of the N4 lone-pair electron density for conformer 1 of Ia on the plane of the 1,2,4-triazolyl ring.

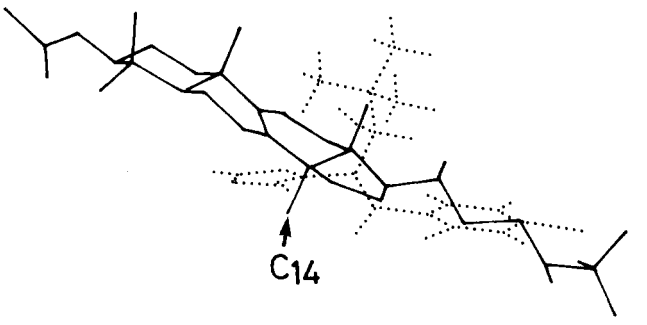

Figure 7. Superimposition of Ia (conformer 1) on lanosterol by ACACS: lanosterol, solid line; Ia (conformer 1), dotted line.

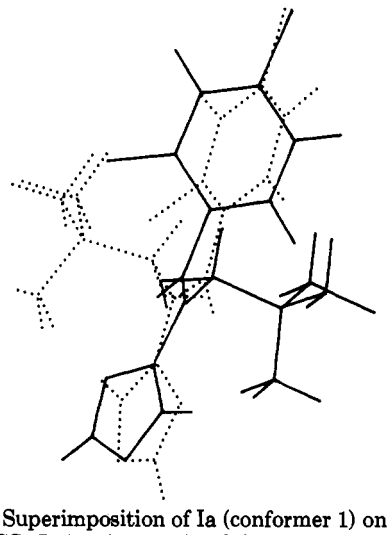

Figure 8. Superimposition of Ia (conformer 1) on Ib (conformer 1) by ACACS: Ia (conformer 1), solid line; Ib (conformer 1), dotted line.

N4 lone pair than conformer 2, the conformation of conformer 1 was used to elucidate the inhibition mechanism of ergosterol biosynthesis by Ia. Figure 7 shows how conformer 1 of Ia is superimposed on the lanosterol derivative. The other superimpositions by computer graphics showed a poor overlapping of two molecules. The N4 atom of the 1,2,4-triazolyl moiety of conformer 1 of Ia has a proximate location to the C14 methyl group of lanosterol, and the hydrophobic phenyl ring occupies an approximately common space with the side chain of the lanosterol derivative, as reported by Marchington (1983). Conformer 1 of Ia was also superimposed on conformer 1 of Ib or IIa to demonstrate the difference of absolute configuration at

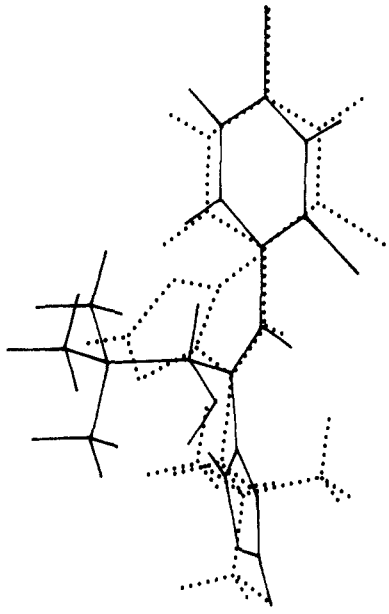

Figure 9. Superimposition of IIa on Ia (conformer 1) by ACACS: Ia (conformer 1), solid line; IIa, dotted line.

C22, which might cause the different interaction with cytochrome P-450. The results are shown in Figures 8 and 9.

DISCUSSION

As Ia has three bulky substituents connected to the olefinic C=C double bond, the freedom of internal rotations is restricted. Meanwhile, the delocalization of π-orbitals in the phenyl, vinyl, and triazolyl groups can stabilize the structure. Therefore, the stabilizing effect and the steric hindrance between the two rings are important factors to determine the dominant conformation. In many cases the conformation of a chemical in solution is estimated by measurement of the coupling constants of proper protons with ^{1}H NMR and subsequent analysis by a Karplus equation (Anderson et al., 1984). However, Ia does not have such a proper proton in the molecule. Therefore, the conformation was quantum chemically estimated based on the ^{1}H NMR spectra at varying temperatures and concentrations (Takasuka and Matsui, 1979) and IR spectra. The results of spectroscopic measurements show that the hydrogen bond is formed between the hydroxy proton and the N2 atom of the 1,2,4-triazolyl ring. Their proximity of the optimized conformation of Ia (N2–H25 = 2.50 Å) and the electron distribution of the N2 lone pair toward the hydroxy group, as shown in Figure 6, support the formation of the intramolecular hydrogen bond.

The active sites of enzymes, which catalyze the 14-demethylation reaction in the ergosterol biosynthesis, have not been clarified in a molecular level. However, the optimized molecular geometries of Ia and its related compounds could be used to discuss their binding to the enzyme active site on the basis of the following reasons. The conformation of Ia is not significantly changed at the enzyme active site because of its rigid molecular shape. The polarity of the active site is similar to that of octanol (Stryer, 1965), of which the dielectric constant (10.3) is actually close to that of chloroform (4.8) used in ^{1}H NMR study. On the basis of these considerations, it seems reasonable that the estimated molecular geometry of conformer 1 of Ia is also realized at the active site of cytochrome P-450 enzymes.

The inhibition of the cytochrome P-450 dependent 14-demethylation of lanosterol is known to proceed by the tight coordination of substrates such as amines to the iron atom of the prosthetic porphyrin (Gadher et al., 1983). I, a racemic mixture of Ia and Ib, showed the type II spectrum, which indicated the binding of I to cytochrome P-450 enzymes via the N4 lone pair of the 1,2,4-triazolyl moiety. The distribution of the N4 lone-pair electron in Figure 6 and the superimposition of conformer 1 of Ia on lanosterol in Figure 7 support this type of binding in the active site, which may lead to the inhibition by Ia.

It is difficult to suppose that the difference of the fungicidal activity between Ia and Ib (Sasaki, 1985) is caused by the difference of the electron density of the N4 lone pair, because there is an insignificant difference between two optical isomers, as listed in Table I. When the intramolecular hydrogen bond was formed between the N2 lone pair and the hydroxy proton, the *tert*-butyl groups of conformer 1 of Ia and Ib oriented in the opposite direction from the plane produced by the phenyl and the 1,2,4-triazolyl ring (Figure 8). This difference of configuration may affect the ability of binding to the active site of enzymes. However, a racemic mixture showed the stoichiometric binding to rat liver microsomal enzymes, suggesting that Ia and Ib were similarly bound to the enzymes. Wiggins and Baldwin, (1984) reported that the binding ability of a fungicide to rat liver microsomes cannot always reflect its fungicidal activity.

The substrate difference spectrum of microsomal enzymes in the presence of II showed a type I spectrum, which indicated the absence of hemochrome formation. In general, the chemical showing a type I spectrum is known to be loosely bound to the cytochrome P-450 and to alter the hydrophobicity near the sixth ligand of porphyrin moiety (Schenkman, 1970). The difference of configuration between IIa and conformer 1 of Ia was apparent by computer graphics. IIa could be fairly well superimposed on conformer 1 of Ia as the *tert*-butyl group of IIa located in the same region of the 1,2,4-triazolyl moiety of conformer 1 of Ia (Figure 9). Supposing that I and II are bound to the same type of cytochrome P-450, the *tert*-butyl group of IIa may locate in the proximity of the iron atom. The loose binding of IIa to the prosthetic porphyrin moiety via the *tert*-butyl group may lead to a type I spectrum.

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Registry No. Piniconazole, 83657-24-3; oxygenase, 9037-29-0; cytochrome P450, 9035-51-2.

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Formation of Pyrazines from Acyloin Precursors under Mild Conditions

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Alkylpyrazines are formed in reactions of acyloins and ammonia under mild conditions and acidic pH. Results from model systems suggest that pyrazines of biogenic origin can be explained by nonenzymic reactions between products of cell metabolism and ammonia.

Alkylpyrazines have been widely investigated as flavor-important trace components in foods. The exact origin of pyrazines remains a mystery, but many model studies suggest that they are minor products of the Maillard reactions of free amino acids and reducing sugars (Maga, 1982). The most prevalent alkylpyrazines, e.g., 2,5-dimethylpyrazine, can reasonably be explained as minor products of the Strecker reaction of amino acids and carbohydrate-derived reductones. The subject of pyrazine formation under relatively high temperature conditions is still under active investigation, and several comprehensive reviews have already appeared (Ohloff et al., 1985; Vernin and Metzger, 1981).

Less attention has been directed toward the chemistry of pyrazine production under biosynthetic conditions. Several reports have appeared describing the natural occurrence of simple pyrazines (McIver and Reineccius, 1986; Gallois, 1984; Kempler, 1983). Tetramethylpyrazine (TMP) formation in bacteria (Demain et al., 1967) has been described as an artifact resulting from mutation-induced dysfunction of *reductoisomerases* in the normal biosynthesis of valine from pyruvate. Thus, α-acetolactate, the normal condensation product of pyruvate, accumulates and eventually undergoes loss of carbon dioxide via *acetolactate decarboxylase* to yield an acyloin, 3-hydroxy-2-butanone (acetoin). Further, it has been suggested that acetoin reacts with ambient ammonia to produce TMP (Kosuge et al., 1971; Demain et al., 1967) although the latter reaction has not been shown to be enzyme catalyzed. Recently, complex alkylpyrazine mixtures resembling those produced in Maillard reactions were reported in fermented cacao (Barel et al., 1985; Gill et al., 1984) and fermented soya products (Liardon and Ledermann, 1980) and in cheese (Liardon et al., 1982). The formation of complex pyrazine mixtures under fermentative conditions can be explained by invoking nonenzyme-catalyzed reactions of a series of biochemically derived acyloins with ammonia. In theory, acyloins are biochemically available from bimolecular reactions of α-keto acids and/or other bioavailable carbonyl compounds. For example, in isoleucine biosynthesis (White et al., 1968) α-ketobutyrate and acetaldehyde combine to form α-aceto-α-hydroxybutyrate, which on decarboxylation could yield another acyloin, 3-hydroxy-2-pentanone.

The purpose of this study was to examine the nature of pyrazine formation from acyloins and ammonia under mild

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