methylase activity but did not affect aniline hydroxylase or microsomal protein and cytochrome b_5 and P-450. This observation agreed with that of Eling, *et al.*[6] except they stated that DPH, 75 mg/kg injected intraperitoneally twice a day for 3 days, increased P-450 content. DPH did not increase the spectral change of P-450 with hexobarbital, a type I substrate, but increased significantly the N-demethylation of aminopyrine, also a type I substrate. The spectral change with aniline was increased by DPH but the hydroxylation of aniline was not enhanced. These facts are not interpreted only by the higher affinity of aniline ($Km=10^{-5}M$) than that of aminopyrine ($Km=10^{-4}M$).[8,15] Some other factors must be taken into consideration.

The effect of DPH on the microsomal phospholipid content and on their fatty acid compositions differes markedly from those of PB or ethanol and/or ethionine. PB increased the total phospholipid content being due to the increase of PE and PC, but DPH increased significantly only PE content and resemble to ethanol and/or ethionine. However, ethanol or ethionine enhanced only apparent aniline hydroxylase activity and increased the percentage of polyunsaturated fatty acids in PE fraction.[4,5] On the contrary, DPH enhanced only aminopyrine demethylase activity and decreased the percentage of unsaturated fatty acids in PE or PC fraction. Minor phospholipids, such as sphingomyelin and phosphatidylinositol, seemed not to be so much affected by these agents.

The important roles of phospholipids in the hepatic microsomal oxidation have been ascertained by several investigators, Strobel, *et al.*,[16] Chaplin, *et al.*.[17] It is not obvious how the changes in the composition of phospholipids in microsomes relate to the drug metabolism, but DPH might change the conformation and function of endoplasmic reticulum and alter apparent enzymatic activity.

15) H. Remmer, R.W. Estabrook, J. Schenkman and H. Greim, *Arch. Exptl. Pathol. Pharmakol.*, **259**, 98 (1968).
16) H.W. Strobel, A.Y.H. Lu, J. Heidema and M.J. Coon, *J. Biol. Chem.*, **245**, 4851 (1970).
17) M.D. Chaplin and G.J. Mannering, *Mol. Pharmacol.*, **6**, 631 (1970).

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Conformational Analysis of 1,3-Diethyl-5-methyl-5-nitrohexahydropyrimidine

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Two research groups[2,3] have determined activation energies for the ring inversion of simple hexahydropyrimidines by the nuclear magnetic resonance (NMR) studies at low-temperature. Before those works, Urbanski and his co-workers[4] have investigated some 5-methyl-5-nitrohexahydropyrimidines and tentatively concluded that the 1,3-diequatorial

1) Location: *a) Mejirodai, Bunkyo-ku, Tokyo; b) Shirogane, Minato-ku, Tokyo.*
2) F.G. Riddel and J.M. Lehn, *Chem. Commun.*,**1966**, 375; F.G. Riddel, *J. Chem. Soc.* (*B*), **1967**, 560.
3) R.F. Farmer and J. Hamer, *Tetrahedron*, **24**, 829 (1968).
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and 5-nitro axial conformation is favoured for N-benzyl and N-cyclohexyl substituents. This paper describes the conformational equilibrium of 1,3-diethyl-5-methyl-5-nitrohexahydropyrimidine (I) by the measurements of dipole moment and low-temperature NMR studies aiming to find any clue on the conformation of antimicrobial 5-amino-1,3-di-*n*-alkyl-5-methylhexahydropyrimidines.[5]

TABLE I. Dipole Moment Data of 1,3-Diethyl-5-methyl-5-nitrohexahydropyrimidine (I)

ω_2[a]	ε_{12}	v_{12}
0.00667	2.3541	0.6281
0.01426	2.4728	0.6311
0.03236	2.7708	0.6373
0.03771	2.8696	0.6391
0.06867	3.3948	0.6503

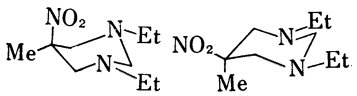
ε_1[b]	v_1	d ε_{12}/d ω_2	d v_{12}/d ω_2	$P_{2\infty}$	P_E	μ
2.2380	0.6258	16.74	0.3560	409.4	54.3	4.12

a) The symbols used are: subscripts 1,2 and 12 refer to solvent, solute and solution, respectively; ε, dielectric constant; v, specific volume; $P_{2\infty}$, specific solute polarization at infinite dilution; P_E, solute molar electronic polarization; ω, weight fraction; μ, dipole moment.
b) The values of ε_1, v_1, d ε_{12}/d ω_2 and d v_{12}/d ω_2 were determined graphically.

The dipole moment of I, which has been synthesized by Senkus,[6] was measured in CCl_4 solution at 20° to be 4.12 D. The data are given in Table I. We assume that I exists as a mixture of two conformers (IA) and (IB), which are shown in Fig. 1 with their expected moments calculated by Piotrowska, *et al.*[4] The conformer IA bears 1,3-diequatorial and 5-nitro axial conformation, while the conformer IB bears 1,3-diequatorial and 5-methyl axial conformation. Mole fractions of conformers IA and IB, *i.e.*, N_A and N_B, are calculated from Eq. 1 using the observed moment value:

$$4.35^2N_A + 3.12^2N_B = 4.12^2 \qquad \text{Eq. 1}$$

To solve the equation leads N_A=0.79 and N_B=0.21.



conformers	IA	IB
calculated dipole moments[4]	4.35 D	3.12 D

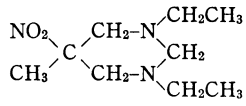
Fig. 1

In order to confirm the assumption that I is in an equilibrium of two conformers, the NMR spectrum of I was taken at 60 MHz by varying temperatures. The spectral data are given in Table II.

As shown in Table II, the spectrum of the ring 2-methylene protons is an AB quartet at room temperature. Upon cooling, the shape of the signal broadens progressively and collapses at *ca.* −45°. At lower temperatures (below −50°), new signals of 2-methylene protons appear as two AB quartets in different intensities. The signal of 4- or 6-methylene protons shows a similar behaviour with that of 2-methylene protons. The signal for 5-methyl group further changes from a singlet at 22° to a doublet in unequal intensities at −63°. The appearance of two sets of signals suggests that there exist two conformers in different amounts with slow inversion at lower temperatures.

5) F.A. Barkley and B.S. Schwartz, *Antimicrobial Agents Ann.*, **1960**, 507 [*Chem. Abstr.*, **56**, 14402f (1962)].
6) M. Senkus, *J. Am. Chem. Soc.*, **68**, 1611 (1946).

Table II. NMR Data of 1,3-Diethyl-5-methyl-5-nitrohexahydropyrimidine (I)



Temp. (°C)	5-CH_3 δ[a]	Ring protons C-CH_2-N δ	C-CH_2-N $\delta\nu_{AB}$	N-CH_2-N δ	N-CH_2-N $\delta\nu_{AB}$	CH_3-CH_2-N CH_3 δ	CH_3-CH_2-N CH_2 δ
+22	1.44s	2.82q	0.73	3.03q	0.34	1.04t	2.37q
−22	1.42s	2.78q	1.06	2.97q	0.54	1.03t	2.34q
−43	1.40bs	collapsed to two broad humps				1.03t	2.32q
−63 major signals	1.35s	2.75q	1.74	2.94q	1.31	1.03t	2.31q
−63 minor signals	1.67s	2.54q	1.22	2.75q	1.06		

a) Chemical shifts (δ) and chemical shift differences ($\delta\nu_{AB}$) between the signals of the axial and equatorial protons for the ring methylene groups are given in ppm. s=singlet, bs=broad singlet, t=triplet, and q=quartet

Farmer and Hamer[3] have studied the NMR spectra of hexahydropyrimidines and hexahydro-*sym*-triazines at low temperature, and described that the chemical shift difference ($\delta\nu_{AB}$) between the signals of the axial and equatorial protons for the N-CH_2-N group lies in the 0.88—1.30 ppm range at *ca.* −60°, when two lone pairs on the nitrogen atoms are diaxial as shown in Fig. 2. In the spectrum of I at −63°, the $\delta\nu_{AB}$ values of the major (1.31 ppm) and minor (1.06 ppm) signals for 2-methylene protons suggest that both major and minor conformers bear the lone pair diaxial conformations, *i.e.*, the 1,3-dialkyl diequatorial conformations. When referred to the dipole moment data, it is obvious that the major signals of ring protons are those of the conformer IA. Thus, the minor signals may be due to the conformer IB. The chemical shift for 5-methyl signal of the conformer IB is in 0.32 ppm down-field than that of the conformer IA. This fact may be explained by assuming that the 5-methyl group is axial and the 5-methyl protons exist in the anisotropic field of the lone pair electrons on the ring nitrogen atom of IB.[7] The signals of the methyl and methylene protons for the N-ethyl group do not coalesce at +22°——63°, which may be due to the similarlities of chemical shifts and coupling constants of the ethyl signals in conformers IA and IB.

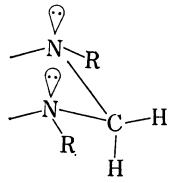


Fig. 2

It is concluded from the observed data that I exists at room temperature as a mixture of two conformers undergoing fast inversion, and that below *ca.* −45° the inversion process becomes so slow on the NMR time scale as the separate conformers are seen. The coalescence temperature is determined from the NMR spectra as −43°. The relative magnitudes of the major and minor signals indicate that the ratio of the conformers IA and IB is 6: 1 at −63°, which implies that N_A is 0.86 and N_B, 0.14. These figures are comparable with those obtained from the dipole moment measurements.

Urabanski, *et al.*[8] have reported in the study of 5-methyl-5-nitrotetrahydro-1,3-oxazines that the N-alkyl and 5-nitro diaxial conformation was favoured for N-*n*-alkyl and N-benzyl

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8) T. Urbanski, D. Gürne, R. Kolinski, H. Piotrowska, A. Jonczyk, B. Serafin, M. Szretter-Szmid, and M. Wintanowski, "Proceedings of the International Symposium on Nitro Compounds," Warsaw, 1963, p. 198.

substituents. By contrast, any considerable amount of the conformer bearing 5-nitro and one N-ethyl diaxial conformation is not detected in I. There might exist considerable differences between tetrahydro-1,3-oxazines and hexahydropyrimidines in the magnitudes of effects, such as the lone pair-lone pair repulsion,[9] 1,3-diaxial alkyl-nitro interaction, and so on. Conformational study on antimicrobial 5-amino-5-methylhexahydropyrimidines[5] are now undertaken.

Experimental

NMR Measurements——A JEOL JNM-C-60H spectrometer equipped with a JES-VT-3 variable temperature probe was used. Spectra were taken at 60 MHz for 10% CS_2 solutions using TMS as an internal standard, and recorded every 3—10 degree over the range between +22° and −63°.

Dipole Moment Measurements——The Dipolemeter FAM-3A (2 MHz) manufactured by Yamato Scientific Instruments Co. was used for the measurements. The dipole moments were measured in CCl_4 solution at 20°. Densities were measured with a pycnometer of *ca.* 5 ml capacity. The electronic polarization of 1,3-diethyl-5-methyl-5-nitrohexahydropyrimidine (I) was obtained by measuring the refractive index of I (n_D^{20} 1.4728). The moments were calculated essentially by the method of Halverstadt and Kumler.[10]

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Synthesis of Norepinephrine-O-sulfates

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In the studies of the metabolism of norepinephrine (NE), norepinephrine-O-sulfates *i.e.* NE-3-sulfate and NE-4-sulfate, were required and the chemical synthesis was undertaken. The synthetic route of the desired NE-O-sulfates from catechol diacetate is outlined in Chart 1.

3,4-Dihydroxyacetophenone (II), 4-benzyloxy-3-hydroxyacetophenone (IIIa), 3-benzyloxy-4-hydroxyacetophenone (IIIb), 4-benzyloxy-α-bromo-3-hydroxyacetophenone (IV) and 4-acetoxy-3-benzyloxy-α-bromoacetophenone (VIII) were prepared by the methods similar to those described by Imai and Tamura[2] and identical with specimens given in literatures.[2,3]

In order to protect the resulting amino groups, amination of IV or VIII was carried out with dibenzylamine on refluxing in anhydrous benzene to give the aminoaryl ketone, 4-benzyloxy-α-N,N-dibenzylamino-3-hydroxyacetophenone (V) or 4-acetoxy-3-benzyloxy-α-N,N-dibenzylamino-acetophenone (IXa).

Because of the less reactivity of hydroxy group of V, N,N-dimethylaniline-sulfur trioxide, a mild sulfating agent, was able to sulfate V only to a small extent. Therefore, sulfation of V was attained with pyridine-sulfur trioxide.[4] IXa was deacetylated by hydrolysis with

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2) K. Imai and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **16**, 1854 (1968).
3) J. Hukki and E. Honkanen, *Acta Chem. Scand.*, **13**, 329 (1959).
4) E.E. Gilbert, *Chem. Rev.*, **62**, 552 (1962).