The effects, in increasing order (pK_a), are as follows: β-pyridyl, XII (11.31) <γ-pyridyl, XIII (10.28)[8] <α-pyridyl, XI (10.18). The β-pyridyl group has the weakest effect, but exerts an effect rather similar to that of the phenyl group (11.41).

Among imidazole cyclizations from N'-(*o*, *m* or *p*-substituted-phenyl)arylamidines, the hydrogen atom in the imidazole ring produced should be favorably located so that their acidities might correlate with the directing effects of substituent groups: the 2-aryl-7-substituted-benzimidazole structures would occur predominantly in the ring closure of N'-(*o*-substituted-phenyl)arylamidines and the 2-aryl-5-substituted-benzimidazole structures in the ring closure of N'-(*p*-substituted-phenyl)arylamidines. In addition, both structures might be involved in the closure of the imidazole ring of N'-(*m*-substituted-phenyl)arylamidines, no matter what the potential tautomeric structure *meta* to a substituent group might be.

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8) T. Hisano and M. Ichikawa, *Chem. Pharm. Bull.* (Tokyo), **22**, 1923 (1974).

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Interaction of Chlorpromazine-Hydrochloride with Lecithin Vesicles detected by the Use of Carbon-13 Nuclear Magnetic Resonance

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The interaction of chlorpromazine-HCl with lecithin vesicles was investigated by carbon-13 nuclear magnetic resonance spectroscopy. Signal broadening of the drug induced by its addition to a vesicle–water (D_2O) solution indicated the incorporation of the drug into the vesicle membrane. In the absence of the drug, two resolved signals arising from the carbonyl carbons of the external and internal layers of the vesicle were observed with 0.3 ppm shift difference, but the difference became almost undetectable on addition of the drug. This phenomenon suggests that the incorporated chlorpromazine was located near the carbonyl carbons in the vesicle membrane. Displacement of a trivalent cation from the membrane surface by chlorpromazine-HCl was confirmed by the observation that the upfield shift of exterior choline methyl carbons initially induced by the addition of a shift reagent, Yb^{3+}, to the vesicle solution was canceled out when a sufficient amount of the drug was added to the sample solution.

Keywords——chlorpromazine-hydrochloride; lecithin vesicles; drug interaction; ^{13}C NMR study; shift reagent

Previously we investigated the interaction of a tranquilizer, chlorpromazine-HCl, with lecithin vesicles by proton magnetic resonance (^1H NMR) spectroscopy to obtain fundamental information on the drug interaction with biological membranes. We reported that chlorpromazine-HCl interacted with the polar part of the vesicle membrane surface, displacing the divalent ion Mn^{2+} and trivalent ion Eu^{3+}.[2] In this study, we have analyzed the same inter-

1) Location: *5 Nakauchicho, Misasagi, Yamashina-ku, Kyoto, 607, Japan.*
2) K. Kitamura, T. Takahashi, K. Hozumi, and T. Sato, *Chem. Pharm. Bull.* (Tokyo), **26**, 256 (1978).

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action, employing carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy to confirm the results of the ^1H NMR study and to detect further details of the interaction, since the ^{13}C NMR spectrum of lecithin vesicles in water (D_2O) gives much more highly resolved signals than the ^1H NMR spectrum.

Experimental

Lecithin——Egg yolk lecithin (E. Merck) was purified by column chromatography.[2]

Preparation of Lecithin Vesicle Solution——To *ca.* 120 mg of suitably dried lecithin, one ml of D_2O was added, and the lecithin–D_2O mixture was shaken until it became a white milky dispersion. It was then ultrasonicated for 15—20 min in an ice-water bath under a nitrogen stream so that the sample became an almost transparent solution. The solution was centrifuged at 3500 rpm for 15 min to eliminate any possible sediment stripped from the metal surface of the sonication tip.

Measurements of ^{13}C NMR——^{13}C NMR spectra were measured in an 8 mm tube at *ca.* 38° using a Varian CFT-20 pulse Fourier transform NMR spectrometer (20 MHz). The spectral width was 4000 Hz, and 10000—20000 transients were accumulated in 8K data points.

Results and Discussion

A ^{13}C NMR spectrum of lecithin vesicles in D_2O is shown in Fig. 1a. When the carbonyl region of Fig. 1a was expanded (Fig. 2a), two resonances separated by a 0.3 ppm shift difference, the larger at lower field and the smaller at higher field, were seen. Yeagle *et al.*[3] assigned the lower field signal to overlapping α and β chain carbonyl carbons of lecithin at the external layer of the vesicle and the higher field one to lecithin at the internal layer of the vesicle, using some lanthanide ions (Yb^{3+}, Ho^{3+}, Gd^{3+}), since they do not penetrate the vesicle and induce a shift or broadening of the signals of the carbon nuclei to which they have access. Figure 2b shows the same region of the lecithin vesicle after

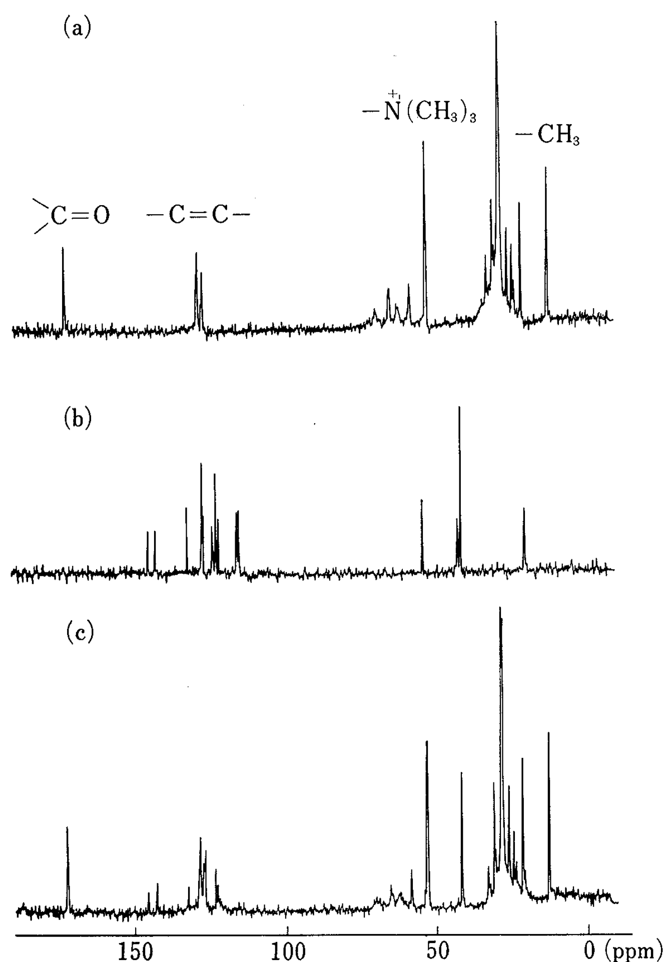

Fig. 1. ^{13}C NMR Spectra of Lecithin Vesicles and Chlorpromazine-HCl

(a): Lecithin vesicles in D_2O.
(b): Chlorpromazine-HCl (85 mM) in D_2O.
(c): After addition of chlorpromazine-HCl (85 mM) to (a).

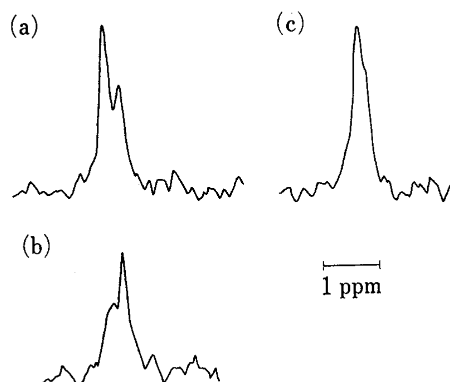

Fig. 2. Expansion of the Carbonyl Region in the ^{13}C NMR Spectra of Lecithin Vesicles

(a): In D_2O (expansion of Fig. 1a).
(b): After addition of Gd^{3+} (0.1 mM) to (a).
(c): after addition of chlorpromazine-HCl (85 mM) to (a) (expansion of Fig. 1c).

3) P.L. Yeagle and R.B. Martin, *Biochem. Biophys. Res. Commun.*, **69**, 775 (1976).

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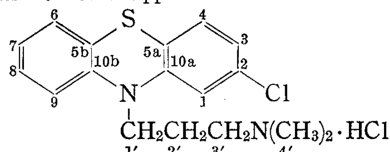
addition of a very small amount of Gd^{3+} ions (0.1 mM). The lower field signal assigned to external carbonyl carbons was broadened and decreased in height, while the higher field one assigned to internal carbonyl carbons was not affected at all. At higher levels of Gd^{3+} (8 mM), the lower field signal disappeared due to extreme broadening.[3]

A ^{13}C NMR spectrum of chlorpromazine-HCl in D_2O is depicted in Fig. 1b. The assignment listed in Table I was carried out from published data for promazine in $CDCl_3$[4] together with a consideration of the Cl substituent effect, taking account of the results of off resonance decoupling and selective decoupling. Chemical shifts in D_2O were measured from the internal reference, dioxane, and are represented in tetramethylsilane scale.

Table I. ^{13}C NMR Chemical Shifts of Chlorpromazine-HCl

Carbon	Shift	
	in D_2O	in $CDCl_3$
1	117.5	116.4
2	133.8	133.3
3	124.4	123.5
4	128.9	128.1
5a	124.4	124.3
5b	125.5	125.5
6	128.4	127.7
7	123.4	122.8
8	128.9	127.7
9	116.9	116.2
10a	146.8	146.1
10b	144.3	143.6
1′	44.5	44.3
2′	22.2	21.7
3′	56.0	55.2
4′	43.5	42.7

Shifts are shown as ppm from TMS.



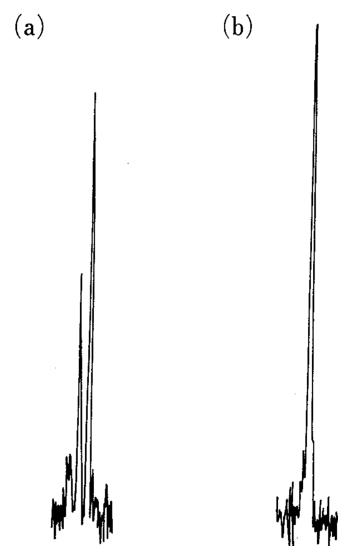


Fig. 3. ^{13}C NMR Spectra of the Choline Methyl Carbons of Lecithin Vesicles

(a): with 20 mM Yb^{3+}.
(b): after addition of chlorpromazine-HCl (110 mM) to (a).

Figure 1c shows a spectrum of chlorpromazine-HCl in a vesicle solution. Chlorpromazine-HCl was added as a dry powder to the vesicle solution to avoid a dilution effect, and the spectrum was taken after the drug had completely dissolved. All signals from the drug, especially protonated ring carbons such as C-1 or C-9, were broadened and decreased in height. This suggested that the mobility of the chlorpromazine molecule was restricted by its penetration into the vesicle membrane. As for the vesicle signals, the shift difference of the carbonyl carbons (0.3 ppm) observed before the drug addition (Fig. 2a) was effectively reduced and became almost undetectable, as shown in Fig. 2c. This seemed to be attributable to the change in the environment of the carbonyl carbons as a result of the approach of the incorporated chlorpromazine.

When Yb^{3+}, which is the most efficient shift reagent for ^{13}C NMR,[5] was added to the vesicle solution, the choline methyl carbon signal of the external surface of the vesicle was shifted to higher field so that the choline methyl signal split into two peaks, as shown in Fig. 3a. An addition of chlorpromazine-HCl (110 mM) to this solution canceled out the induced upfield

4) G. Fronza, R. Mondelli, G. Scapini, G. Ronisivalle and F. Vittorio, *J. Mag. Reson.*, 23, 437 (1976).
5) B. Sears, W.C. Hutton and T.E. Thompson, *Biochemistry*, 15, 1635 (1976).

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shift and the separated signals were merged into the original single signal (Fig. 3b). This phenomenon is consistent with the results of our previous ^{1}H NMR study using Eu^{3+} and Mn^{2+},[2] and displacement of the cation from the vesicle membrane by chlorpromazine-HCl was thus confirmed in ^{13}C NMR spectroscopy.

Recently, Frenzel *et al.*[6] investigated the unsonicated dispersion prepared from a mixture of dipalmitoyl phosphatidylcholine (DPP) and chlorpromazine-HCl (2:1) with 50% water content by means of calorimetry, ^{13}C NMR and ^{31}P NMR, and they reported that chlorpromazine-HCl induced a higher fluidity in the fatty-acid chain region and reduced the mobility of the polar headgroup of DPP. They accounted for their observations by means of a model in which the drug was incorporated into the DPP bilayer, so that the dialkylaminoalkyl chains are located near the polar headgroups and the ring system does not penetrate far beyond the glycerol backbone into the hydrocarbon phase. In our study, the interaction of chlorpromazine-HCl with lecithin was observed by simple addition of the drug to a preformed vesicle–water (D_2O) solution. The ^{13}C NMR spectroscopic results for our sample system confirm that the chlorpromazine molecule penetrated into the vesicle membrane and induced displacement of the trivalent ion Yb^{3+} from the membrane surface. Taking account of the results of Frenzel *et al.*[6] the reduction of shift difference between the two carbonyl carbons induced by the drug addition implied that the chlorpromazine molecule reached a position near the carbonyl carbons which are adjacent to the hydrocarbon chain region.

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6) J. Frenzel, K. Arnold and P. Nuhn, *Biochem. Biophys. Acta*, **507**, 185 (1978).

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