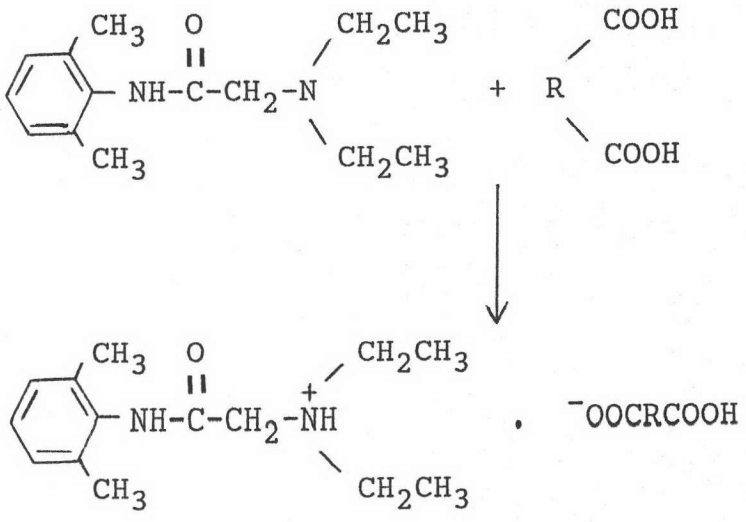
CHAPTER IV

DISCUSSION

The syntheses of organic salts of lidocaine were accomplished in this study. They were lidocaine adipate (IV-A), lidocaine maleate (IV-B), lidocaine malonate (IV-C) and lidocaine tosylate (IV-D). Since the synthesized compounds were easily ionized, they were regarded as prodrugs of lidocaine. Enhancement of skin permeability via ion-pair approach was proposed. The determination of skin permeability and apparent partition coefficients were therefore performed to evaluate the assumption of the enhanced skin permeability of these compounds by ion-pair transportation.

Lidocaine (I) was prepared from lidocaine hydrochloride (monohydrate) by alkaline extraction. Four organic salts of Lidocaine (IV) were synthesized by the reaction of lidocaine and organic acids namely adipic acid, maleic acid, malonic acid and p-toluene-sulfonic acid, respectively. The syntheses of these compounds were shown in Scheme 3 - 4.

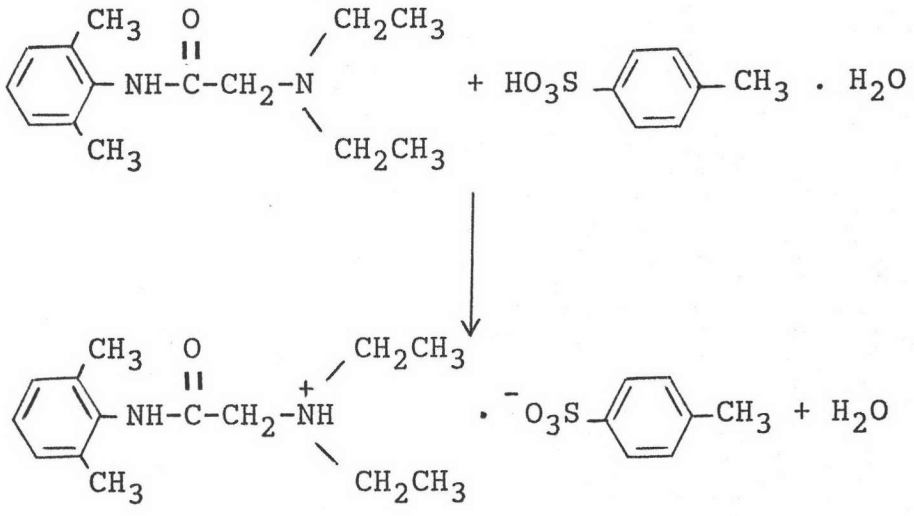


IV-A R = $(CH_2)_4$

IV-B R = HC = CH

IV-C R = CH_2

Scheme 3



Scheme 4

The products were recrystallized from ethyl acetate and the yields were about 70%. As salts, the products obtained had higher melting points than lidocaine and lidocaine hydrochloride. Approximate solubilities of the synthesized compound (Table 3) showed that they were easily dissolved in water (required less than 10 parts of water for 1 part of compound) but very slightly soluble or insoluble in ether. The solubility property was more similar to lidocaine hydrochloride (I.HCl) than lidocaine. However, there were still some differences of solubilities among these four salts. The salts were classified into 2 subgroups by solubility differences. The subgroup of lidocaine maleate (IV-B) and lidocaine tosylate (IV-D) were insoluble in ether which resulted from the low dissociation constant (pK_a) of acidic part of these compounds (Table 21). Another subgroup of lidocaine adipate (IV-A) and lidocaine malonate (IV-C), the increase in solubility of these compounds in non-polar solvent such as ether was found. It was due to lipophilic alkyl chain in acidic part of them. The solubility properties indicated that polarity of IV-A and IV-C were between I, the least polar, and I.HCl, IV-B and IV-D, the more polar molecules.

Ultraviolet absorption spectra of lidocaine adipate (IV-A) and lidocaine malonate (IV-C) in isotonic phosphate buffer pH 7.4 were similar to those of lidocaine (I) and lidocaine hydrochloride (I.HCl) in both pattern and wavelength of maximum absorbance. It can be concluded that the carbonyl chromophore of carboxylates had minor effect on the ultraviolet absorbance spectra of IV-A and IV-C. The molar absorptivity of these compounds were about 460. For lidocaine maleate (IV-B),olefinic structure conjugated with two carbonyl groups in maleate salt shifted the wavelength of maximum absorbance from 262.5 nm to 270.2 nm. The pattern of ultraviolet absorption spectrum altered and the molar absorptivity (ϵ) increased accordingly. Benzene ring in lidocaine tosylate (IV-D) caused changes in the pattern of ultraviolet absorption spectrum of this compound from that of I but the wavelength of maximum absorption was remained at 262.5 nm. Molar absorptivity of IV-D was higher than molar absorptivity of I, 792 versus 463.

The ^{1}H NMR spectra of the products (IV) showed characteristic proton peaks of lidocaine and organic acid in the molecule (Table 6). NMR spectrum of lidocaine adipate (IV-A) showed peaks of β and α $-CH_2$ at 1.62 and 2.20 and peak of dicarboxylic proton of adipic acid at 10.40 ppm, exchangeable with D_2O. For lidocaine maleate (IV-B), peaks of olefinic proton

(=C-H) appeared at 6.29 ppm. Only one proton of carboxylic of maleic acid was found as very broad peak at 13.10 ppm, exchangeable with D_2O. Dicarboxylic protons of malonic acid in lidocaine malonate (IV-C) showed D_2O exchange peak at 11.87 ppm. A broad peak, exchangeable with D_2O at 9.47 ppm of lidocaine tosylate (IV-D) indicated quarternary ammonium proton (N-H) in the compound which was due to strong acidity (low pK_a) of p-toluenesulfonic acid. Acidic proton of p-toluenesulfonic acid was more capable to donate and attach to N-amine of lidocaine. The NMR spectrum of IV-D confirmed that this salt was more stable in form of ion-pair. The chemical shifts of protons on C-atoms attached to both sides of N-atom of amine concluded that strength of $\overset{+}{N}H$ bond between lidocaine and salt of IV-D was the strongest (Table 21). The chemical shifts of $N-CH_2CH_3$ and $CO-CH_2-N$ of IV-B (3.33 and 4.25 ppm) and IV-C (3.19 and 4.14 ppm) were in downfield region when compared with 2.65 ppm for $N-CH_2CH_3$ and 3.22 ppm for $CO-CH_2-N$ of lidocaine. These resulted from quarternary ammonium proton existing in the molecule in the same pattern as IV-D. Peaks of dicarboxylic protons of IV-A, IV-B and IV-C were found to vary from 10.40 ppm of IV-A to 11.87 ppm of IV-C and to 13.10 ppm for IV-B. This was due to variation in pK_a of the acids. The lower in pK_a of the acid indicated that the acid was able to donate proton more easily, such as maleic acid when compared with

malonic acid and adipic acid. Thus, the acidic proton of IV-B and IV-D had more deshielding effect on N-amine and proton on C-atom attaching to N-atom than acidic protons of IV-A and IV-C. Hence, the organic acid which formed salt with lidocaine had effect on the chemical shift of protons of $N-CH_2CH_3$ and $CO-CH_2-N$ of lidocaine. The values of chemical shift related to the pK_a value of the acid part, the lower in pK_a of acid, the more deshielding effect on the protons (Table 6, 21).

The infrared absorption spectra of the synthesized products showed $\overset{+}{N}-H$ stretching at about 2650 - 2500 cm^{-1} for lidocaine adipate (IV-A), lidocaine maleate (IV-B) and lidocaine malonate (IV-C) and at 2850 cm^{-1} for lidocaine tosylate (IV-D) which confirmed the formation of quarternary ammonium salt.

Determination of apparent partition coefficients were carried out to evaluate lipophilicity of sythesized products. The experimental system was octanol : isotonic phosphate buffer pH 7.4. The test compounds; lidocaine hydrochloride (I.HCl), lidocaine adipate (IV-A), lidocaine maleate (IV-B), lidocaine malonate (IV-C) and lidocaine tosylate (IV-D) were added in isotonic phosphate buffer phase while lidocaine (I) was added in octanol phase. The mixture systems were shaken to reach a distribution equilibrium. The concentration of the test compounds

in separated aqueous phase (buffer) were determined by ultraviolet spectrophotometry. The results of apparent partition coefficient (P) in Table 20 showed that P of I was the highest (about 1900), while P of I.HCl, IV-A and IV-C were of the same order of magnitude (P = 60). Low values of P of IV-B and IV-D were found. The results indicated that the more lipophilicity of the molecules together with the higher value of pK_a of IV-A and IV-C made them partitioned into the oil phase (octanol) better than IV-B and IV-D.

In vitro skin permeation study was performed by using a system consisting of a permeation cell and pig skin. The four synthesized compounds(IV),lidocaine (I) and lidocaine hydrochloride (I.HCl) were prepared as 1% w/v solution in propylene glycol. The test solution was applied to the donor cell at 48 hours after preapplication leach period. After application of test compound, the sample from receptor cell was taken and replaced by fresh buffer at the time 12, 24, 36 and 48 hours. The sample solutions taken at various time intervals were quantitated by using first derivative UV spectrophotometry. This method was used to determine the amount of test compounds and was found to minimize the interferences from endogeneous substances from pig skin. Ultraviolet absorption spectrum (Figure 29) showed that interferences from pig skin gave high value of absorbance in the

wavelength range of analysis. Thus, the use of normal mode of ultraviolet spectrophotometry for analysis in this study was impossible according to large and unstable value of the interferences. Besides, the absorbance value of interferences was more than ten times the value of the test compound permeated through pig skin to receptor phase. The technique of first derivative UV spectrophotometry was able to minimize the matrix interferences. D_1 spectrum of skin interferences (Figure 30) showed low and stable value but the D_1 value of skin interferences was not zero (Table 9). However, the percent recovery of the analysis by D_1 was acceptable, within 5% of the added amount. Percent recovery were improved to be less than 2% off the added value by using corrected D_1. The corrected D_1 was used in the analysis of permeated test compound in this study.

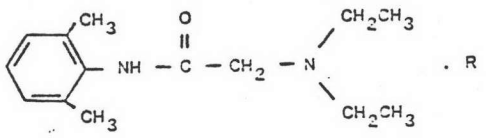
Cumulative amount (Q) and flux (J) of the test compounds; lidocaine (I), lidocaine hydrochloride (I.HCl), lidocaine adipate (IV-A), lidocaine maleate (IV-B), lidocaine malonate (IV-C) and lidocaine tosylate (IV-D) at observed time intervals; 12, 24, 36 and 48 hours were determined. Since the compounds were expected to give pharmacological action in the form of lidocaine, the result can not be compared on the µg basis. The Q and J value were converted to µMole for comparisons. The permeation result in

Table 14-17 showed that at 12 hours after application (Table 14), IV-A and IV-C were not significantly different from I but they gave higher value of Q and J than I.HCl, IV-B and IV-C (significantly different, $\alpha < 0.05$). After 24 hours application, Q and J value of I were the highest whereas IV-A and IV-C lay midway in the range. I.HCl, IV-B and IV-D gave low Q and J value. The permeability profile (Figure 31-38) of synthesized organic salts differed from base by showing rapid transportation at 12 hours. The Q and J of salts declined after 24 hours. The permeability profiles of the salts may be resulted from mechanism of transportation other than passive diffusion. The transportation of the salt may be in ion-pair fashion as proposed which resulted in the improvement of permeability at the first 12 hours after application. As organic salt prodrug, the permeability was enhanced from I.HCl. Nevertheless the permeability was still below I.

The results of permeation study were in agreement with apparent partition coefficient. Lidocaine (I) with the highest partition coefficient penetrated through pig skin better than other test compounds. When the length of alkyl chain of organic acid was considered in case of IV-A and IV-C, it was found that the longer alkyl chain, the higher lipophilicity of molecule and the higher skin

permeation occured. It was due to the low values of both pK_a and chemical shifts (ppm) of protons of $N-CH_2-CH_3$ and $CO-CH_2-N$ (Table 21). These values were related to the ability to form ion-pair. For this reason, more stable ion-pairs of IV-B and IV-D were found and skin permeability was found to be enhanced, in spite of poor lipophilicity. It can be concluded that both lipophilicity and pK_a of acid part contribute the important roles in the transportation of lidocaine organic salts via ion-pair mechanism.

Table 21 : Comparison of properties of test compounds.



Test Compound[a]	R	Permeability(Q)[b]		Partition Coefficient		Aqueous Solubility	^{1}H NMR(ppm)		pK$_a$[c] of R
		12 hr.	24 hr.	P	log P		N-CH_2CH_3	CO-CH_2-N	
I	-	4.98	10.81	1918.90	3.28	slightly soluble	2.65	3.22	7.7[d]
I.HCl	HCl	0.42*	4.14*	64.35	1.81	freely soluble	2.60	3.22	-7.0
IV-A	$(CH_2CH_2COOH)_2$	2.87#	7.35*#	62.63	1.80	freely soluble	2.65	3.20	4.41, 4.43
IV-B	$(CHCOOH)_2$	1.89*	4.43*	25.96	1.41	very soluble	3.33	4.25	1.83, 6.07
IV-C	$CH_2(COOH)_2$	3.29#	5.18*	58.95	1.77	very soluble	3.19	4.14	2.83, 5.69
IV-D	$CH_3C_6H_4SO_3H$	2.13*	4.45*	1.28	0.11	freely soluble	3.34	4.36	-1.34

[a] I = lidocaine, I.HCl = lidocaine hydrochloride, IV-A = lidocaine adipate, IV-B = lidocaine maleate, IV-C = lidocaine malonate, IV-D = lidocaine tosylate.

[b] Cumulative amount of test compound (μM) permeated at T hours after application.

[c] Weast, R.C. (1974).

[d] pK$_a$ of lidocaine (Bokesch, Post and Strichartz, 1986).

* Significantly different ($\alpha < 0.05$) when compared with I.

Significantly different ($\alpha < 0.05$) when compared with I.HCl.