Antimicrobial activity was not activated by N-substitution. When the structure of AB ring in steroids was 3,5-diene or 5-ene, the effect of substituents was to keep or weaken the activity of original compounds, and magnitudes of cativity were in the order of $CH_2CH_2CN > (CH_2$-$CH_2OH)_2 > (CH_2CH_2Cl)_2 \gg (CH_2)_3NH_2$. The compounds derived from inactive XVII were all inactive, therefore, in 5-en-3β-ol no activation effect was observed.

Substitution of aminopropyl group to 17β-aminosteroids brought VII, XIV, and XIX marked anti–inflammatory action.

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The Relationship between the Hydroxylated Position of Aromatic Compounds by Monooxygenase and Various Electronic Reactivity Indices

It has been recognized that a large number of both normally occurring and foreign aromatic compounds are hydroxylated by monooxygenase in the animal body to form phenolic compounds. However, little information is available concerning the biochemical mechanisms involved, and the extent of hydroxylation and orientation of substituting group have been the subjects of a vast number of studies with intact animals.

At this time, if a common theory by which hydroxylation reaction of aromatic compounds by monooxygenase may be illustrated uniformly at an electronic level were to be presented, it would be very important to clarify the reaction of monooxygenase and also useful to predict the structure of hydroxylated compounds of various drugs. In this work reported here, the π-electron densities and free valence numbers in the case of ground and first electronic excited state were obtained for some of aromatic substances by a molecular orbital method, simple LCAO MO method for π-electrons.

The parameters of coulomb and resonance integrals for substituent groups are the same as the values cited in previous papers.[1,2]

These results are shown in Fig. 1. In this respect, the π-electron density is a measure of nucleophilic and electrophilic reactions and the free valence number is a measure of radical reaction.

1) S. Hata, M. Yamakawa, S. Tomioka and H. Sano, *Chem. Pharm. Bull.*, (Tokyo), **13**, 96 (1965).
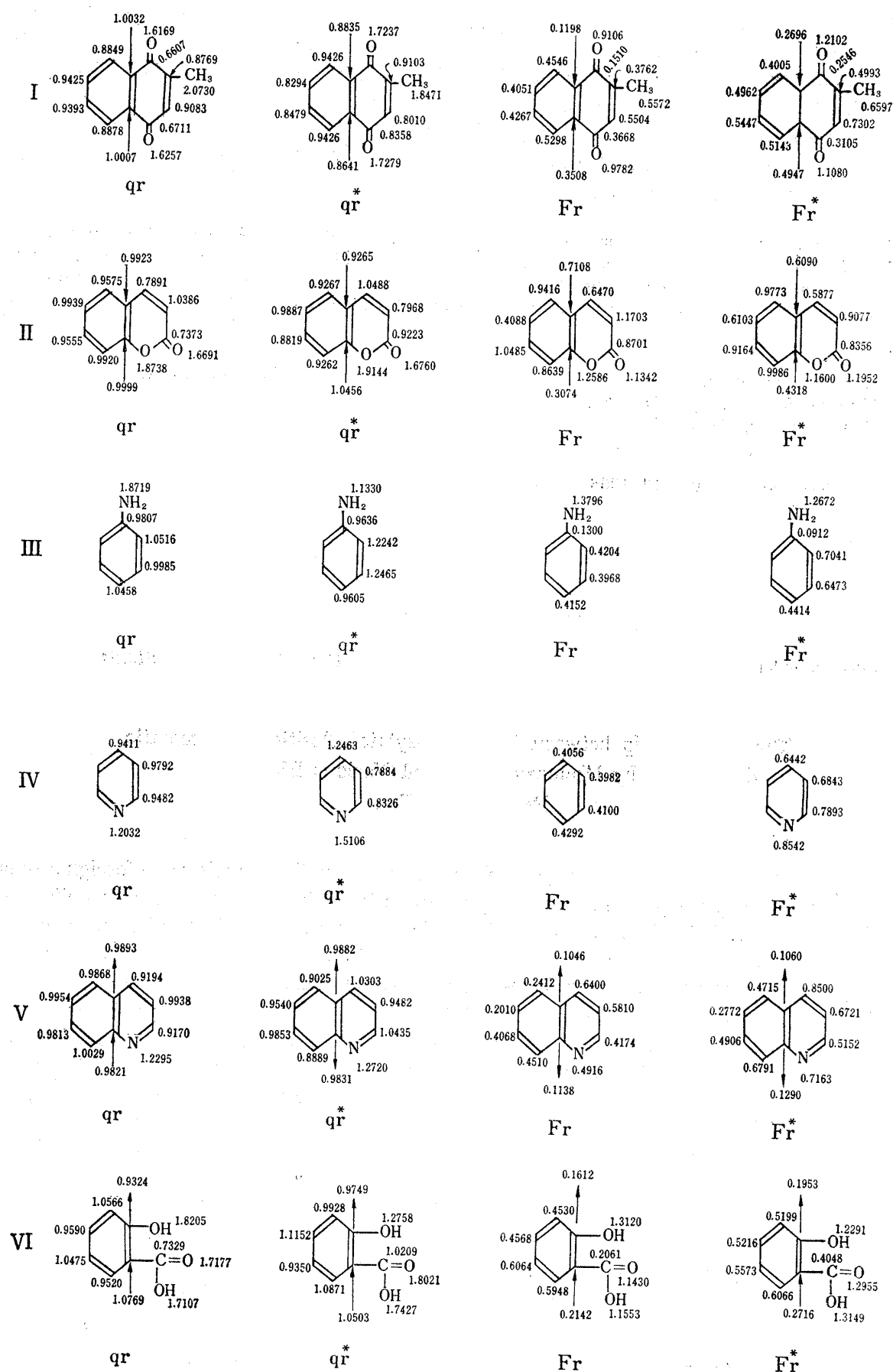2) S. Hata, K. Mizuno and S. Tomioka, *Yakuzaigaku*, **27**, 130 (1967).
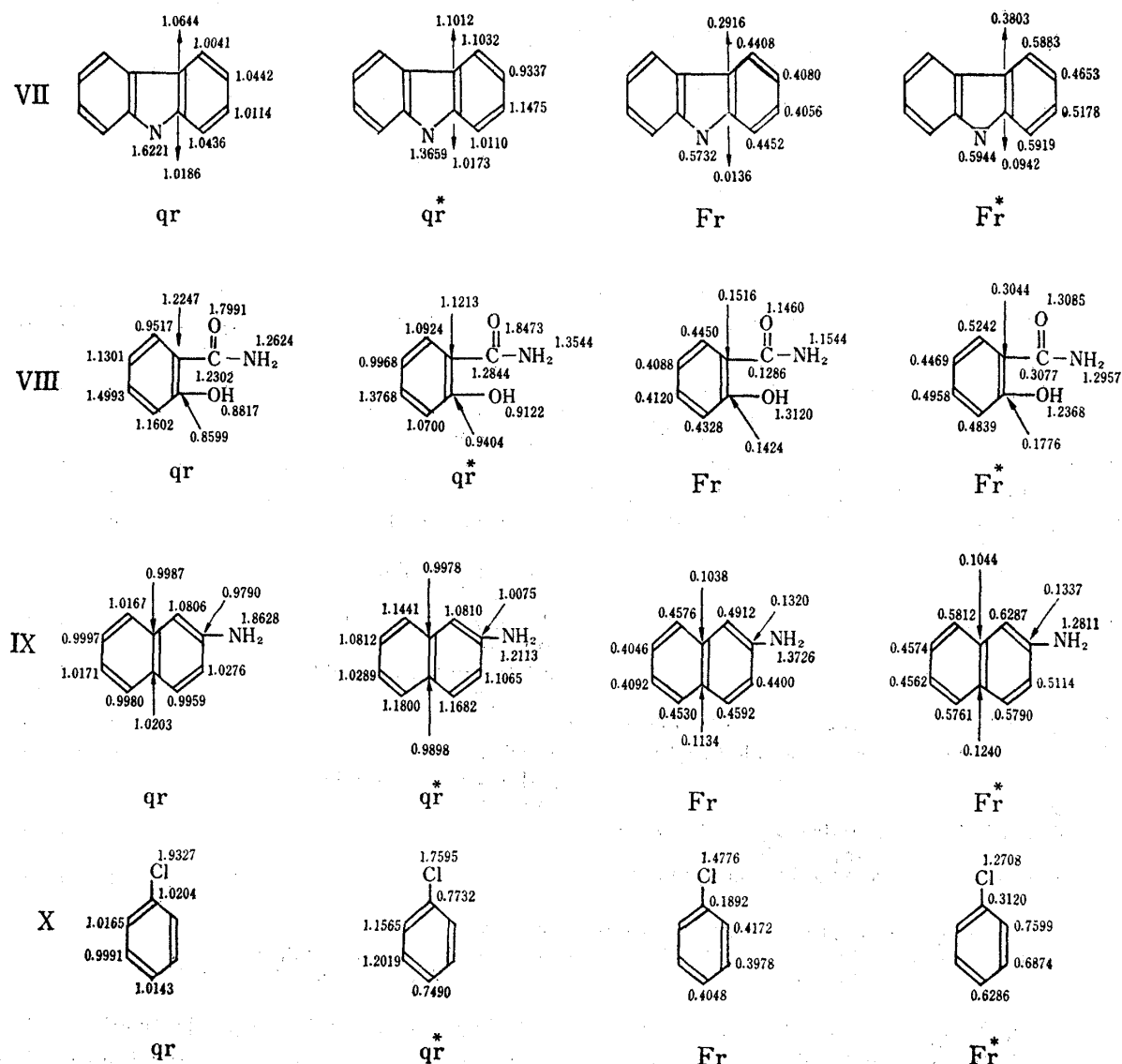
Fig. 1 (continued)

Fig. 1. Various Reactivity Indices

qr: π-electron density in the ground state   qr*: π-electron density in the first excited state
Fr: free valence number in the ground state   Fr*: free valence number in the first excited state

Subsequently, relationship between the hydroxylated position of these aromatic substances[3-13] and the π-electron density or the free valence number in the case of ground and first electronic excited state was investigated. From the results summarized in Table I, it should be noted that the mechanism of hydroxylation reaction of these aromatic substances by monooxygenase cannot be considered as the nucleophilic reaction in the ground state, electrophilic

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10) S.E. Wright, *Experientia*, **18**, 416 (1962).
11) R.T. Williams, "Detoxication Mechanisms," Chapman & Hall, London, 1959. p. 359,
12) E. Boyland, *Biochem. J.*, **69**, 601 (1958).
13) J.N. Smith, *Biochem. J.*, **47**, 284 (1950).

reaction in the first electronic excited state, or the radical reaction either in the ground state or in the first electronic excited state. Further, electrophilic reaction in the ground state also cannot explain the results of these hydroxylation reactions completely. On the other hand, nucleophilic reaction in the first electronic excited state can explain these results admirably.

TABLE I. Relationship between Hydroxylated Position
of Aromatic Compounds and Reactivity Indices[a,b]

No.	Compound	Hydroxylated compound Main product	By–product	Ground state E N R			First excited state E N R		
I	Menadione	phthiocol (rat, rabbit)[3]	——	×	×	○	×	○	○
II	Coumarin	3-hydroxycoumarin(rabbit)[4] 7-hydroxycoumarin (man)[5]	7-hydroxy coumarin (rabbit)[4]	×	×	○	×	○	×
III	Aniline	p-aminophenol (rabbit)[6]	o-aminophenol (rabbit)[6]	○	×	×	×	○	×
IV	Pyridine	3-hydroxypyridine (rabbit)[7]	——	○	×	×	×	○	×
V	Quinoline	3-hydroxyquinoline(rabbit)[8]	6-hydroxyquinoline (rabbit)[8] 8-hydroxyquinoline (rabbit)[8]	○	×	×	×	○	×
VI	Salicylic acid	gentisic acid (man)[9]	2,3-dioxybenzoic acid (man)[9] 2,3,5-trioxy benzoic acid (man)[9]	○	×	×	×	○	×
VII	Carbazole	3-oxycarbazole (rabbit, rat)[10]	——	○	×	×	×	○	×
VIII	Salicylamide	gentisic amide (rat)[11]	gentisic amide (man)[11] 2,3-dioxybenzoic amide (man)[11]	×	×	×	×	○	×
IX	β-Naphthylamine	5-hydroxy β-naphthylamine (rat, rabbit)[12]	——	×	×	×	×	○	×
X	Chlorobenzene	p-chlorophenol (rabbit)[13]	——	×	×	×	×	○	×

a) E: electrophilic reaction; N: nucleophilic reaction; R: radical reaction
b) ○ show the case that the result of hydroxylation reaction can be explained as E, N, or R, respectively.
× show the case that the result of hydroxylation reaction cannot be explained as E, N, or R, respectively.

Hence, it may be concluded that some nucleophilic reactions in the first electronic excited state of substrate seem to play an important role in the mechanism of hydroxylation reaction of aromatic substances by monooxygenase, and in this connection, it may be assumed that O_2^- [14,15] as a nucleophilic reagent seems to participate in these reactions. The present results show that π-electron density in the first electronic excited state will in future become an indicator for predicting the hydroxylated compounds of some kinds of aromatic substances.

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