Retention Mechanism of Imidazoles in Connective Tissue. III.[1] Aldehyde Adduct Formation of a 4(5*H*)(or 5(4*H*))-Imidazolone Product *in Vitro*

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2-Methylimidazole (2MI), as well as imidazole, has been thought to undergo cupro-ascorbate (Cu-VC)-catalyzed oxidative transformation *in vitro* to become a reactive species capable of combining with aldehydes intrinsic to connective-tissue proteins. We attempted to seize the essence of the above reaction through obtaining the structural information of an aldehyde-bonding species. As major products from 2MI in the *in vitro* Cu-VC system, 2-hydroxymethylimidazole (2(OH)MI) and 2-methyl-4(5*H*)(or 5(4*H*))-imidazolone (2MIone) were identified by mass-spectral and chromatographic comparison with the corresponding authentic standards synthesized. The *in situ* addition of acetaldehyde or propionaldehyde as a simple protein-aldehyde model to the system resulted in the deducible formation of an aldol condensate, 2-methyl-4(or 5)-ethylidene-4(5*H*)(or 5(4*H*))-imidazolone (2MEIone) or its possible analogue with a propylidene moiety, respectively. The authentic compound of 2MIone directly reacted with acetaldehyde and easily afforded the products assignable to the isomers of 2MEIone through the ethylidene moiety at physiological pH and temperature, whereas neither 2MI or 2(OH)MI reacted at all. These results suggest that a 4(5*H*)(or 5(4*H*))-imidazolone product, although simply a monooxygenated form, is sufficiently reactive to give aldol condensation-typed covalent adducts with aldehydes, even under physiological conditions, probably having an activated methylene moiety in the ring structure. Based on the present results, we discussed the mechanism of the retention of imidazole-containing drugs in connective tissue.

Key words imidazolone; covalent binding; aldehyde adduct; connective tissue

The five-membered heterocyclic imidazole structure is found in a large number of drugs or drug candidates.[2] It has been reported that, for several imidazole-containing drugs, the marked retention of their equivalents in connective tissue was observed after dosing to laboratory animals.[3—6] However, the mechanism of the retention, which potentially involves some toxicological significance, remains unclear.

Investigations to elucidate this mechanism have been conducted in our laboratory using radiolabeled compounds with a simple structure: imidazole (Im) and its 2-methyl derivative (2MI). A previous study in rats has indicated that the *in vivo* retention of imidazole-containing drugs is largely attributable to irreversible binding between the imidazole moiety and elastin, a major macromolecule of the extracellular matrix, and that their interaction might be mediated through a cytochrome P450-independent biotransformation of drugs with an imidazole moiety.[7]

In an *in vitro* study, it has also been indicated that the catalytic oxidation of the cupro-ascorbate (Cu-VC) system can be involved in the activation of both Im and 2MI to give their reactive species capable of combining with aldehydes intrinsic to connective-tissue proteins.[1] These reactions occurred even under physiological conditions.

In the present study, ^{14}C-labeled 2MI as a model substrate was reacted in the Cu-VC system, and structural elucidation of its products was undertaken, especially focusing on identification of an aldehyde-bonding species.

MATERIALS AND METHODS

Chemicals [Ring-C2-^{14}C] 2MI (51.2 μCi/μmol), synthesized by Chemsyn Science Laboratories (Kansas City, KS, U.S.A.), was purchased from Nemoto & Co., Ltd. (Tokyo). Its radiochemical purity was assessed as being more than 97% by TLC analysis. The specific activity of the incubation mixtures was adjusted through dilution with unlabeled 2MI, obtained as having more than 98% purity from Wako Pure Chemical Industries, Ltd. (Osaka). The incubation mixtures were prepared in double-distilled water to minimize metal contamination.

The following reference compounds for analytical use were prepared synthetically in our research center, and their structural analysis was performed by ^{1}H-NMR spectrometry and thermospray liquid chromatography/tandem mass spectrometry (TSP LC/MS/MS).

2-Methyl-4(5*H*)(or 5(4*H*))-imidazolone (2MIone) was obtained by reacting ethyl acetimidate (Sigma, St. Louis, MO, U.S.A.) with glycine methyl ester (Wako), as previously reported by Jacquier *et al.*[8] ^{1}H-NMR (dimethyl-d_6 sufoxide (DMSO-d_6)) δ: 2.02 (s, 3H, CH_3), 3.89 (s, 2H, CH_2), 10.77 (broad s, 1H, NH). TSP-MS *m/z* (rel. int. %): 99 (MH^+, 100).

2-Hydroxymethylimidazole (2(OH)MI) was synthesized from imidazole-2-carboxaldehyde (Fluka, Buchs, Switzerland) through a crossed Cannizzaro reaction. ^{1}H-NMR (DMSO-d_6) δ: 4.43 (s, 2H, C2-CH_2), 6.89 (s, 2H, C4- and C5-H). TSP-MS *m/z* (rel. int. %): 99 (MH^+, 100). Although the signals for OH and NH protons were not clearly observed in the ^{1}H-NMR spectrum, the presence of alcohol and imidazole moieties in this compound was indicated by MS/MS analysis of the MH^+ ion, which exhibited peaks at *m/z* 81 and 69 corresponding to a dehydrated fragment and a protonated fragment of the imidazole moiety, respectively.

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2-Methyl-4(or 5)-ethylidene-4(5*H*)(or 5(4*H*))-imidazolone (2MEIone) was obtained by reacting 2MIone with acetaldehyde (E. Merck, Darmstadt, Germany) under an alkaline condition. ^{1}H-NMR (DMSO-d_6) δ: 1.97 (d, J=7.3 Hz, 3H, CH-C$\underline{H}_3$), 2.12 (s, 3H, C2-CH_3), 6.19 (q, J=7.3 Hz, 1H, C$\underline{H}$-CH_3), 11.04 (broad s, 1H, NH). TSP-MS *m/z* (rel. int. %): 125 (MH^+, 100). Though not evidently deduced from the above spectra, the possibility that this compound could exist as a mixture of *cis* and *trans* forms in an equilibrium is taken up later.

Deuterium-labeled compounds were purchased from Nippon Sanso Co., Ltd. (Tokyo). All other chemicals and reagents were of the highest purity available and, unless otherwise indicated, were obtained from Wako Pure Chemical Industries, Ltd.

***In Vitro* Reactions** The reaction of [^{14}C]2MI with copper ion and ascorbate was achieved in 0.1 M sodium phosphate buffer (pH 7.2). The standard reaction mixture, in a final volume of 5 ml, contained 1 mM [^{14}C]substrate (2 μCi/ml), 0.05 mM cupric chloride, and 5 mM L-ascorbic acid, and was incubated in a shaking water bath maintained at 37 °C under an aerobic condition up to 24 h. In addition, incubations of the substrate without copper ion and/or ascorbate were performed as controls.

The aldehyde adduct formation of [^{14}C]2MI-derived products in the Cu-VC system was examined as follows. After 5 min of the aforementioned reaction, acetaldehyde or propionaldehyde was added to the mixture at a final concentration of 1 mM, then it was further incubated at 37 °C for 1 h. A control test was conducted similarly, except that buffer alone was added instead of the aldehydes.

The reactivity of either authentic 2MIone, 2(OH)MI, or 2MI to acetaldehyde was examined by direct incubation in 0.1 M phosphate buffer (pH 7.2) at 37 °C for 1 h, each at a concentration of 1 mM.

At pre-determined intervals, aliquots (−50 μl) were withdrawn from the incubates and immediately injected into analytical instruments.

Instrumentation HPLC was performed on a Gilson model 305 system (Middleton, WI, U.S.A.) equipped with a Raytest Ramona 93 radioisotope detector (Straubenhardt, Germany) and a Linear 206PHD UV detector (Reno, NV, U.S.A.). A C_{18}-silica-packed column (SUMIPAX ODS A212, 5 μm, 6.0 mm i.d. ×150 mm, Sumika Chemical Analysis Service, Osaka) was maintained at 50 °C and subjected to either of the following two gradient elution systems: (A), a mobile phase of water–methanol containing 0.1% (w/v) perfluoroheptanoic acid (Kanto Chemical, Tokyo) as an ion-pairing reagent, a linear ramp from 0 to 13% methanol over 20 min with a hold for 20 min, followed by a further linear ramp up to 50% over 10 min; or (B), a mobile phase of water–methanol, a 5 min hold at 0% methanol, followed by a linear ramp to 15% over 5 min with a hold for 10 min, followed by a rapid rise to 50% with a 5 min hold. Each elution system was operated at a flow rate of 1 ml min^{-1}. For radioactivity quantification, the HPLC effluent was fractionated, mixed with a Flo-Scint II scintillation cocktail (Packard, Downers Grove, IL, U.S.A.), and then analyzed in a Beckman LS6000TA liquid scintillation counter (Fullerton, CA, U.S.A.). The total recovery of chromatographed ^{14}C material was greater than 95% in either case for the elution systems.

TSP LC/MS/MS was performed on a Finnigan MAT TSQ70 triple-stage quadrupole mass spectrometer (San Jose, CA, U.S.A.). HPLC separations were conducted with a Shimadzu LC 9A liquid chromatograph (Kyoto) under the same conditions as those previously described, and a post-column addition of 0.6 M ammonium acetate to the mobile phase was performed for the efficient ionization of analytes by using a Yokogawa LC100 solvent delivery system (Tokyo) at a flow rate of 0.2 ml min^{-1}. Detailed operating conditions were as follows: ionization mode, TSP (filament off, discharge off); ion source temperature, 250 °C; vaporizer temperature, 85 °C; repeller voltage, 20 V; collision gas, argon at a pressure of 10^{-3} Torr; collision energy, −40— −60 eV.

^{1}H-NMR spectra were recorded at 200 MHz with a Varian VXR-200 instrument (Palo Alto, CA, U.S.A.). DMSO-d_6 was used as a solvent, and chemical shifts are reported in ppm (δ) downfield from the internal standard, tetramethylsilane.

RESULTS

HPLC Profile of 2MI-Derived Products in a Cu-VC System Products from [^{14}C]2MI in the *in vitro* Cu-VC system were analyzed by the radio-HPLC with the aid of a counterion effect (elution system A). As shown in Fig. 1, this metal/reductant-dependent catalytic reaction led to four peaks of major products, with retention times of 5, 13, 17, and 21 min (P_1—P_4 in the elution order). Immediately after the reaction was initiated by the addition of ascorbic acid to the incubation mixture, P_1, P_3, and P_4 were detectable (−2%

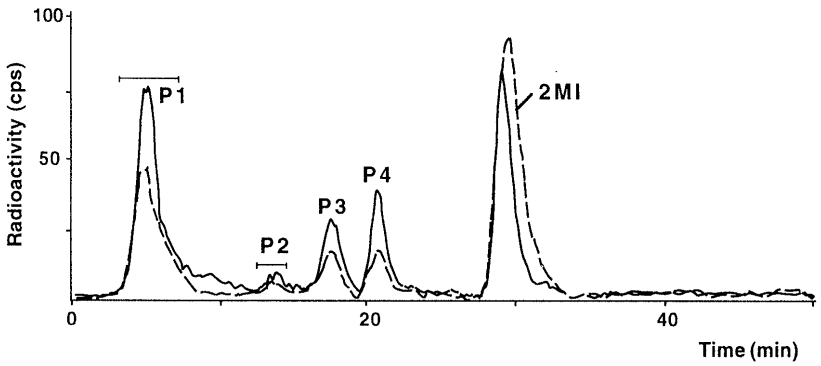

Fig. 1. Representative Radio-HPLC Profiles of [^{14}C]2MI-Derived Products in a Cu-VC System

HPLC separation was conducted on a C_{18} column eluted with gradient system A as described in Materials and Methods. Dashed and solid lines indicate the profiles of 1- and 24-h-reacted mixtures, respectively. Four peaks of major products with retention times of 5, 13, 17, and 21 min (P_1—P_4 in the elution order) were observed.

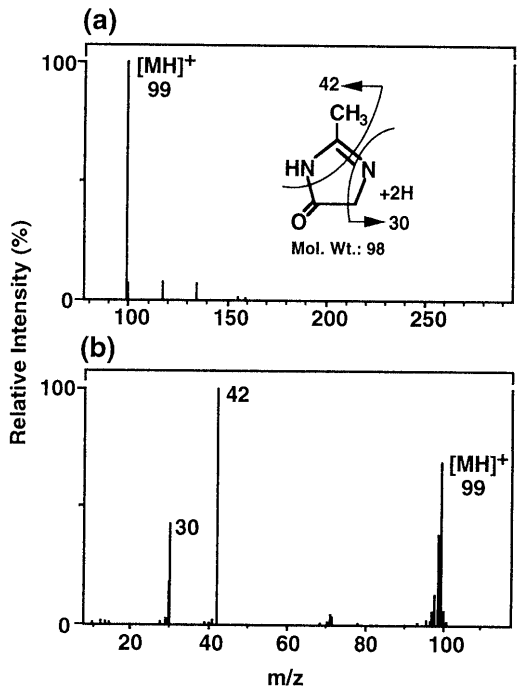

Fig. 2. Mass-Spectrometric Observation of P_3

(a) Mass spectrum of P_3. (b) Product ion mass spectrum of *m/z* 99 from P_3 (collision energy: −40 eV).

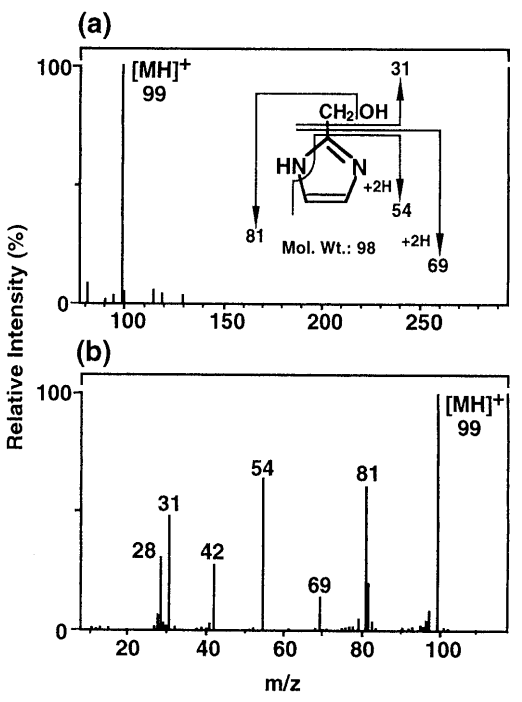

Fig. 3. Mass-Spectrometric Observation of P_4

(a) Mass spectrum of P_4. (b) Product ion mass spectrum of *m/z* 99 from P_4 (collision energy: −40 eV).

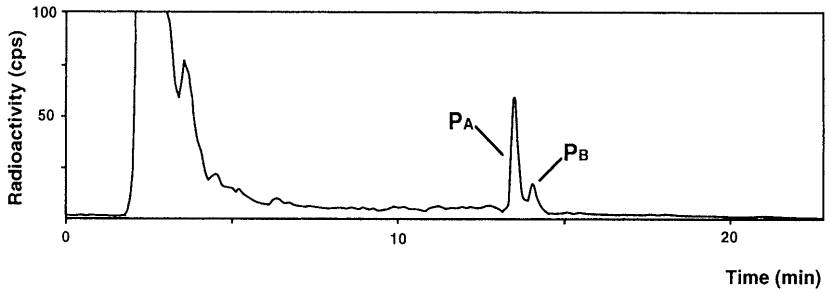

Fig. 4. Radio-HPLC Detection of [^{14}C]2MI-Derived Aldehyde Adducts in a Cu-VC System

HPLC separation was conducted on a C_{18} column eluted with gradient system B as described in Materials and Methods. Two peaks with retention times of 13.5 and 14.0 min (P_A and P_B in the elution order) were observed.

of total ^{14}C chromatographed). Also, P_2 was detectable after 5 min of the reaction. A gradual increase in the amount of the products was observed during the course of incubation, and their formation almost reached a plateau after 4—8 h of incubation. After 24 h, −68% of the substrate was converted, and the P_1—P_4 accounted for −44, 5, 16, and 19% of the total products formed, respectively. Further, the control incubation samples lacking copper ion and/or ascorbate gave only some minor peaks other than the substrate with a baseline resolution on HPLC even after 24 h. This confirms that 2MI is chemically stable under noncatalytic conditions.

Structural Information of 2MI-Derived Products Obtained by TSP LC/MS/MS For P_3 and P_4, each mass spectrum displayed a protonated molecular ion at *m/z* 99, thus indicating the addition of one oxygen atom to the parent molecule of 2MI (Figs. 2a and 3a). Collisional activation of the 99 ion of P_3 gave fragment ions at *m/z* 42 (base peak) and 30 (intensity of 42.0% of the base peak) (Fig. 2b). On the other hand, for the collisional activation of the 99 ion of P_4, the fragments contained a dehydrated peak at *m/z* 81 (intensity of 61.1% of the base peak at *m/z* 99) (Fig. 3b), suggesting of the presence of an alcohol moiety. Authentic 2MIone or 2(OH)MI gave an identical mass fragmentation and retention time on HPLC with those of P_3 or P_4, respectively. As to P_1 and P_2, their mass-spectrometric information was not sufficient for structural characterization by reason of the presence of a large amount of interfering ions, probably derived from ascorbate or its degraded compounds.

Trapping of a Reactive Species with Aldehydes To detect a reactive species in the products of [^{14}C]2MI, either acetaldehyde or propionaldehyde as a simple protein-aldehyde model was added to the reaction mixture of the Cu-VC system, then adduct formation was traced by the radio-HPLC with elution system B. As shown in Fig. 4, the addition of acetaldehyde resulted in the formation of two peaks, P_A and P_B, with retention times of 13.5 and 14.0 min, respectively. Their combined production accounted for −6% of the total radioactivity chromatographed. No such peaks were observed in the absence of the aldehyde. The mass spectrum of P_A displayed a protonated molecular ion at *m/z* 125, 42 mass units greater than that of 2MI (Fig. 5a), and the product ion mass spectrum of the 125 ion showed fragment ions at *m/z* 42

(base peak) and 56 (intensity of 11.1% of the base peak) (Fig. 5b). This was also true of P_B. It was plausibly suggested that the products have carbonyl and ethylidene moieties in their molecules, and that they are isomeric with each other. The possible presence of an ethylidene moiety was also supported by an experiment with deuterium-labeled acetaldehyde (2, 2, 2-d_3 form), in which the incorporation of d_3 into the products, suggestive of no enamine formation with a secondary amine, was observed (mass spectra not shown). Further, when the ^{14}C products on HPLC were carefully fractionated to be uncontaminated with each other, processed to freeze-drying or evaporation at 50 °C *in vacuo*, and subjected to re-chromatography under the same conditions, each gave two peaks with the same retention times as those of the origin again. The reference 2MEIone also gave two peaks on HPLC, which were co-eluted with the *in vitro* reaction products, and they were not isolatable through chromatographic fractionation, as is the case with the products. Their mass-spectral data corresponded to those of the products. Williams *et al.* have pointed out that 2-phenyl-4(or 5)-arylidene-4(5*H*)(or 5(4*H*))-imidazolones can exist as a mixture of *cis* and *trans* forms in equilibrium, and that their interconversion can occur by conventional preservation.[9] This nature, thought to be subtly linked with tautomerism, could apply to the case of 2MEIone. Further, when the reference 2MEIone was dissolved in acidic, alkaline, or alcoholic solution, and then analyzed by HPLC/UV (210 nm) with elution system B, the relative abundance of the two peaks varied according to the kinds of solution. As a ^1H-NMR observation for the reference 2MEIone, the methyl proton signals of the ethylidene part and of the ring C-2 position tended to decrease in intensity with time after the addition of deuterium oxide to the solvent of DMSO-d_6. Taking these data and the information of analogous compounds into account, P_A and P_B are assignable to isomers of 2MEIone, and there is a likelihood of involvement of geometrical isomerism through an ethylidene moiety, though the preferential configuration under this experimental condition was difficult to clarify. Besides, when propionaldehyde was added to the reaction mixture in the Cu-VC system, the probable formation of an aldol condensate, 2-methyl-4(or 5)-propylidene-4(5*H*)(or 5(4*H*))-imidazolone (involving two possible isomers through propylidene moiety) was indicated by LC/MS/MS (spectra not shown). Based on these results, 2MIone is considered to be a 2MI-derived reactive species sufficient to form aldol condensation-type covalent adducts with aldehydes.

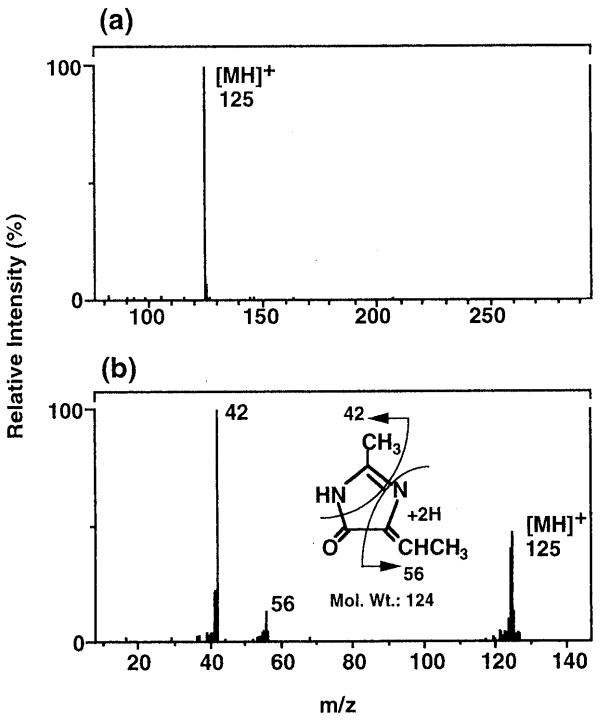

Fig. 5. Mass-Spectrometric Observation of P_A

(a) Mass spectrum of P_A. (b) Product ion mass spectrum of *m/z* 125 from P_A (collision energy: −60 eV).

Reactivity of 2MIone to Acetaldehyde The aldol condensation-type reaction of 4(5*H*)(or 5(4*H*))-imidazolones with aldehydes has been postulated as an intermediate key

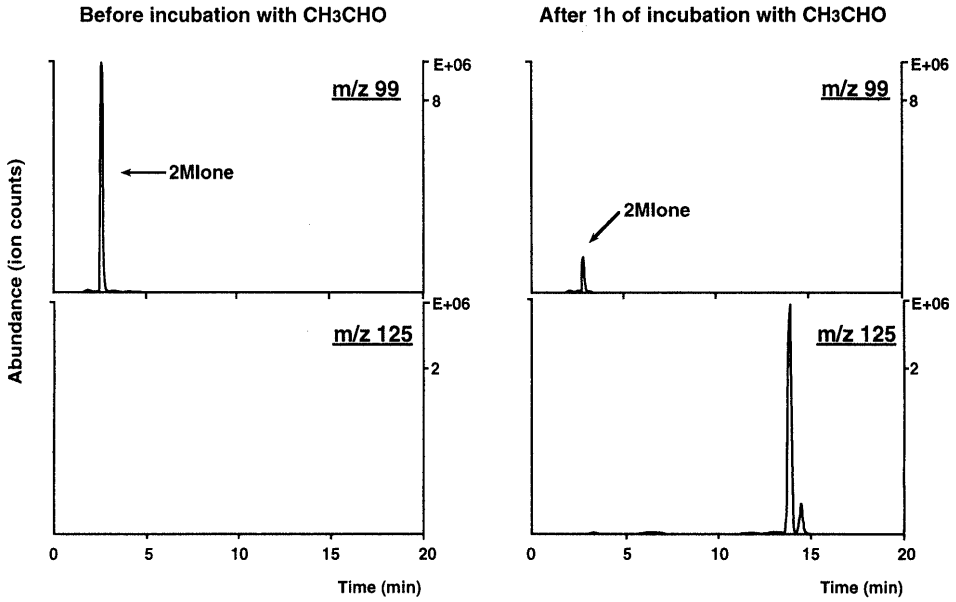

Fig. 6. Reactivity of 2MIone to Acetaldehyde Traced by TSP Ion Chromatography

The substrate 2MIone and reaction products were monitored as ions at *m/z* 99 and *m/z* 125, respectively, under the same separating conditions as those in Fig. 4.

step in the organic synthesis of numerous 2-phenyl-4(or 5)-arylidene-4(5*H*)(or 5(4*H*))-imidazolones.[9,10] As far as we are aware, however, no information is available concerning this reaction under noncatalytic or physiological conditions. Therefore, using the authentic compound of 2MIone, its reactivity to acetaldehyde was directly examined at physiological pH and temperature. As shown in the ion chromatograms (using elution system B) of Fig. 6, after 1 h of incubation with the aldehyde, the formation of two peaks of products monitored at *m/z* 125 was observed with a marked decrease of 2MIone monitored at *m/z* 99 (−12.8% of the initial peak area). There was no detectable peak other than the substrate 2MIone and the products on the total ion monitoring (scanning range: *m/z* 80—400) of this sample. Attempts to isolate either of the products through HPLC fractionation met with failure, and their behaviors on chromatographic and mass-spectral inspection were identical with those of P_A and P_B in Fig. 4, and of the reference 2MEIone, indicating that they are assignable to the isomers of 2MEIone previously described. Besides, when authentic 2MI or 2(OH)MI was incubated with the aldehyde under the same conditions, no product was observed at all.

DISCUSSION

Our experimental results demonstrated that 2MIone, a simply monooxygenated product from 2MI found in the Cu-VC system, can react readily with aldehydic function through aldol-type condensation at the methylene part of the ring C-4(or 5) position, even under noncatalytic and physiological conditions. In the ^{1}H-NMR analysis of the reference 2MIone, the addition of deuterium oxide to the solvent of DMSO-d_6 almost brought about the disappearance of the methylene proton signal (around at 3.9 ppm), so that its portion must be activated to cause a hydrogen-deuterium exchange with ease. This characteristic is probably due to the electron-attracting effect of the neighboring carbonyl group and tertiary nitrogen atom, as previously postulated concerning this class of compounds in the field of organic chemistry.[10] Further, when a preliminary examination using Im as a substrate was performed under the same conditions as those for 2MI, LC/MS/MS data indicated that the addition of acetaldehyde or propionaldehyde to the Cu-VC system resulted in the probable formation of an aldol condensate, 4(or 5)-ethylidene-4(5*H*)(or 5(4*H*))-imidazolone or its analogue with a propylidene moiety, respectively (spectra not shown). This coincidentally suggests that Im-derived nucleophilic 4(5*H*)(or 5(4*H*))-imidazolone was generated similarly in the catalytic system.

A considerable amount of P_1 derived from 2MI was found in the Cu-VC system (Fig. 1), but not enough structural information was available. Because the reaction of N-benzoylhistidine in the same catalytic system can yield some imidazole-ring-ruptured products through a series of free radical reactions,[11] a resemblant pathway may be responsible for the P_1 formation. Besides, P_1 is also separable by HPLC with the aid of a counterion effect of perfluoropentanoic acid, which is able to be distilled away, while P_2, P_3, and P_4 remain in a mixture. The two fractions on the HPLC were obtained from a 4-h-reacted ^{14}C mixture of the Cu-VC system through multiple injections/peak collections, evaporated to dryness, and then subjected to the *in vitro* examination for binding to slices of dog aorta by a previously described method.[1] As a result of autoradiographic detection, the tissue-bound radioactivity was observed for the mixed fraction of P_2—P_4, whereas no radioactivity was observed for the P_1 fraction. We thus think that, although a large amount of P_1 in the Cu-VC system was formed, its fraction is not involved in the binding of 2MI with connective tissue.

It was observed that the catalytic oxidation in the Cu-VC system yields a reactive imidazolone product at physiological pH and temperature. Although in the interest of obtaining a sufficient quantity of the product for structural analysis the concentrations of the catalytic agents were set up high in the present study, our previous study[1] has indicated that the *in vitro* irreversible binding of Im or 2MI equivalents to connective tissue is inducible by the catalysts at concentration levels comparable with their contents of such biological fluids as whole blood, plasma, or serum in human or laboratory animals.[12—14] It is thus conceivable that the catalytic oxidation might also take place to some extent during *in vivo* circulation. We have proposed that, as a possible interpretation of the retention of imidazole-containing drugs in connective tissue, an imidazole nucleus could combine with aldehydic residues found in elastin or collagen through an oxidative form of biotransformation.[1,7] The findings in the present study coincide with and support the above proposal; hence, the depiction of a reaction scheme shown in Fig. 7 might be within the bounds of possibility. It is theoretically supposed that, having no regard for steric hindrance, the reaction pathway expands further to the reductive 1,4-Michael addition of free forms of the nucleophilic imidazolones to an α, β-unsat-

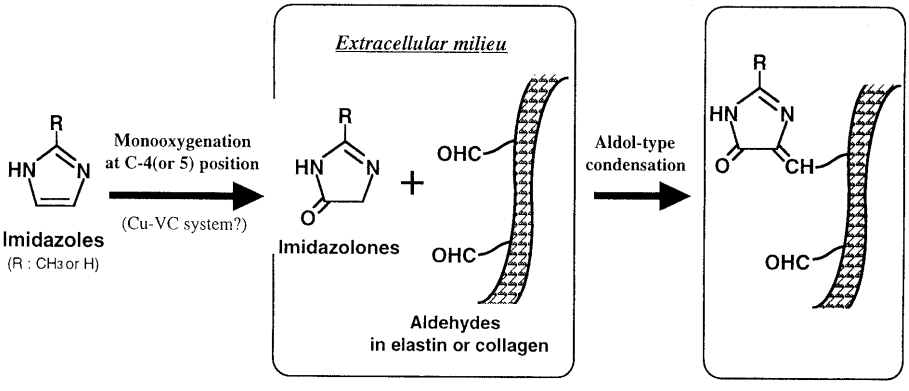

Fig. 7. A Postulated Bio-Reaction Scheme for the Covalent Binding Formation of Imidazoles in Connective Tissue

urated carbonyl system of the ready-built imidazolone-protein adducts. Such a related product could not be detected, however, in the present model experiment.

In the normal construction process of elastin or collagen fibers, the aldehydic function plays an essential role as a basic intermediate of covalent cross-linkages that contribute importantly to elasticity or tensile strength for the respective fibers.[15] Strictly speaking, the biogenic aldehyde comprises the two types of peptidyl residue, namely, allysine (α-aminoadipic acid δ-semialdehyde) and hydroxyallysine (δ-hydroxy, α-aminoadipic acid δ-semialdehyde).[16] The former is biosynthesized from peptidyl lysine and is found in both elastin and collagen,[15] but its contents of these fibrous proteins have been reported to vary and to be elastin>collagen on comparison per 10^3 amino acids.[17] On the other hand, the latter aldehydic compound is biosynthesized from the hydroxylated form of peptidyl lysine, which is found not in elastin but in collagen.[15] Moreover, the aldehydic function of hydroxyallysine has been suggested to be less reactive than that of allysine through the ability to shift to a 5-keto group in a keto-enol mechanism (–CHOH–CHO $\leftrightarrows$ –C(OH)=CHOH $\leftrightarrows$ –CO–CH_2OH).[18] Our previous study has shown that the *in vivo* retention of Im and 2MI equivalents in connective tissue is largely based on their preferential binding to elastin.[7] Consequently, it is reasonable to speculate that some of the imidazoles are more likely to form a covalent adduct with the allysine aldehyde. Additional studies are necessary to investigate this point, in order to discuss the elastic-organ-selective toxic potential for imidazole analogues, which depends on the prevention of the normal allysine-derived cross-linking.

In conclusion, we here present evidence for the aldehyde adduct formation of a 4(5*H*)(or 5(4*H*))-imidazolone product *in vitro*, which might provide a mechanistic explanation for the marked retention of several imidazole-containing drugs in connective tissue. To confirm the authenticity of this explanation, analysis of the biological samples after dosing of imidazole compounds is currently under way in the search for imidazolone-related structures as metabolites (including adducts with endogenous carbonyls).

Acknowledgments The authors are indebted to Mr. Y. Nakamura, Ms. Y. Watanabe, and Ms. H. Inuma for spectral measurement, and to Dr. Y. Ohuchi for provision of the reference compounds.

REFERENCES

1) Ohta K., Akimoto M., Kohno Y., Fukushima K., Suwa T., Awazu S., *Biol. Pharm. Bull.*, **21**, 308—310 (1998).
2) Carganico G., Cozzi P., *Il Farmaco*, **46**, 209—231 (1991).
3) Ostrowski J., Schraven E., Keil M., *Arzneim.-Forsch./Drug Res.*, **26**, 218—225 (1976).
4) Mizojiri K., Kawahara S., Takeuchi M., Inazawa K., Takashima A., Tanaka H., Sugeno K., *Iyakuhin Kenkyu*, **16**, 187—199 (1985).
5) Graham D. J. M., Hama K. M., Smith S. A., Kurz L., Chaplin M. D., Hall D. J., *Drug Metab. Dispos.*, **15**, 565—570 (1987).
6) Yano K., Kozano H., Numata H., Esumi Y., Mitsugi K., Ooyama T., Watanabe I., Wada R., Yatabe N., Ueda T., Sugai S., *Xenobio. Metabol. Dispos.*, **6**, 535—552 (1991).
7) Ohta K., Yamaguchi J., Akimoto M., Fukushima K., Suwa T., Awazu S., *Drug Metab. Dispos.*, **24**, 1291—1297 (1996).
8) Jacquier R., Lacombe J. -M., Maury G., *Bull. Soc. Chim.* (France), 1040—1051 (1971).
9) Williams D. L., Symonds F. L., Ekeley J. B., Ronzio A. R., *J. Am. Chem. Soc.*, **67**, 1157—1159 (1945).
10) Hofmann K., "The Chemistry of Heterocyclic Compounds, Imidazole and Its Derivatives (part I)," ed. by Weissberger A., Interscience Publishers, New York, 1953.
11) Uchida K., Kawakishi S., *Bio-Org. Chem.*, **17**, 330—343 (1989).
12) Sass-Kortsak A., *Adv. Clin. Chem.*, **8**, 1—67 (1965).
13) Spector R., *N. Engl. J. Med.*, **296**, 1393—1398 (1977).
14) Suzuki M., Aoki T., *Pediatr. Res.*, **35**, 598—601 (1994).
15) Stryer L., "Biochemistry," Freeman W. H. and Company, London, 1975, pp. 185—204.
16) Siegel R. C., "International Review of Connective Tissue Research," Vol. 8, ed. by Hall D. A., Jackson D. S., Academic Press, New York, 1979, pp. 73—118.
17) Pinnell S. R., Martin G. R., *Biochemistry*, **61**, 708—716 (1968).
18) Siegel R. C., *J. Biol. Chem.*, **252**, 254—259 (1977).