論　文

ピラン環の開環による縮合型タンニン分子構造の修飾

光永　徹

Structural Modification of Condensed Tannins by the Pyran-Ring Opening

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緒　言

本論文は、樹皮の主要成分である縮合型タンニンの三フッ化ホウ素（BF_3)-フェノール系による新たな分子構造の修飾法と構造解析法について研究し、さらに本修飾法による生成物の特性及び機能を解明することにより、新たな高度利用の可能性を提示したものである。

第1章では、縮合型タンニンの単量体である(+)-catechinをBF_3－フェノール試薬で処理した際に、高収率で生成するピラン環開環型フェノール付加物をはじめ数種の生成物の化学構造を明らかにした。さらに、各種反応溶媒と酸触媒の影響について検討を加え、フェノール付加物の生成がS_N2機構で進行すること、またBF_3の高触媒活性能は反応サイトとのアクセシビリティーの強さに起因することを明らかにした。

第2章では、天然の縮合型タンニンに存在するピラン環の開環度の新解析法としてメチル化－核交換反応を確立した。すなわち、構造既知の合成縮合型タンニンのモデル化合物を用いて本手法によるピラン環構造の定量的解析が可能であることを確認し、これを開環度未知のタンニンに適用したところ、特にケブラチョタンニンではかなりの頻度で開環していることを初めて明らかにした。よって、本分析法は高分子領域も含む天然タンニンのピラン環に関して新たな情報を提供できることが明らかになった。

第3章では、分子量、ピラン環の開環度などの構造解析結果から、樹皮タンニンのフェノール化挙動はflavan-3-ol単位の水酸基パターンによって大きく異なることを明らかにすると共に、フェノール化変性によりA-環の求核性が大きくなり、室温でのホルムアルデヒドとの反応が向上することを示した。また、樹皮タンニンのグルコシルトランスフェラーゼ阻害活性は緑茶及びウーロン茶由来のタンニンのものよりかなり高く、分子量が増加するに従い阻害活性が向上すること、ならびにフェノール化変性物は分子量低下の割には高い阻害活性を維持していることを明らかにした。

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PREFACE

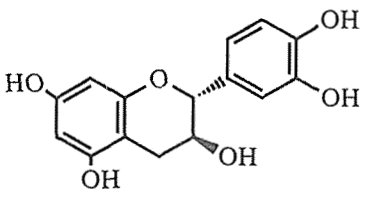
Condensed tannins consist of oligomeric or polymeric flavan-3-ols as repeating units and are widely distributed in the plant kingdom. They have been considered as defensive substances against living-plant enemies.[1-3] Barks of some woody species, *Acacia mearnsii*,[4,5] *Pinus taeda*,[6] *Pinus radiata*[7-9] *Larix* sp.,[10] *Cryptomeria japonica*,[11] and *Quercus falcata*,[12] are known for the sources of condensed tannins. In particular, the bark of hemlock and spruce were the most important materials in America historically, and their industry for tanning of leather had been already operated in the middle of the 19th century.[13]

The word of tannin was used in the 1800's to designate unknown compounds isolated from the tan oak galls that were employed in the tanning of leather. Rosenheim[14] found that condensed tannins have a nature of producing anthocyanidins pigment when treated with acid. Furthermore, Bate-Smith and Swain[15] defined vegetable tannins as water-soluble phenolic compounds having molecular weights between 500 - 3000, and special properties such as the ability to precipitate alkaloids, gelatin, and other proteins.

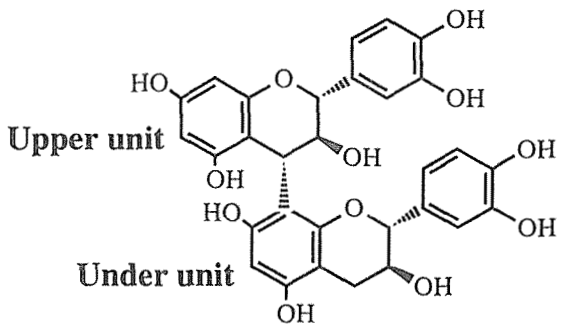
Structural complexities in condensed tannins are centered principally on variations in hydroxylation patterns of the flavan chain extender units, on the stereochemistry at the three chiral centers of heterocyclic ring, and on the location and type of interflavanoid bond.

The condensed procyanidins consisting of 3,3′,4′,5,7-pentahydroxyflavan units are found most widely in plant, and about 50 proanthocyanidins from dimer to hexamer have been reported.[16] Most of the natural compounds isolated in the bark of woody plant have 2*R*,3*R* (2,3-*cis*) chain extender units, and they are terminated with either a (+)-catechin or (-)-epicatechin unit.[17,18] Further most proanthocyanidins are linked simply by bonds between the C4 of the pyran-ring to either the C6 or C8 of the flavan A-ring. In the 5-deoxy profisetinidins and prorobinetinidins, C4 to C6 linkages between the chain extender unit are favored.[19,20] These structural patterns also play an important role in the reactions that occur on the A-ring and B-ring as well as the interflavanoid bond. Many different electrophiles tend to react with the A-ring because of its high electron density, and this electrophilic reaction has some significances with respect to commercial utilization such as wood adhesives. Interflavanoid bond is very labile under acidic conditions as can be seen in the reactions with butanol-HCl[21] and the thiolysis[22-24] which have long been important analytical tools used in determining the structure of polymeric proanthocyanidins. On the other hand, the formation of metal complexes[25] and the antioxidant characteristics[26] of condensed tannins are associated with their B-ring.

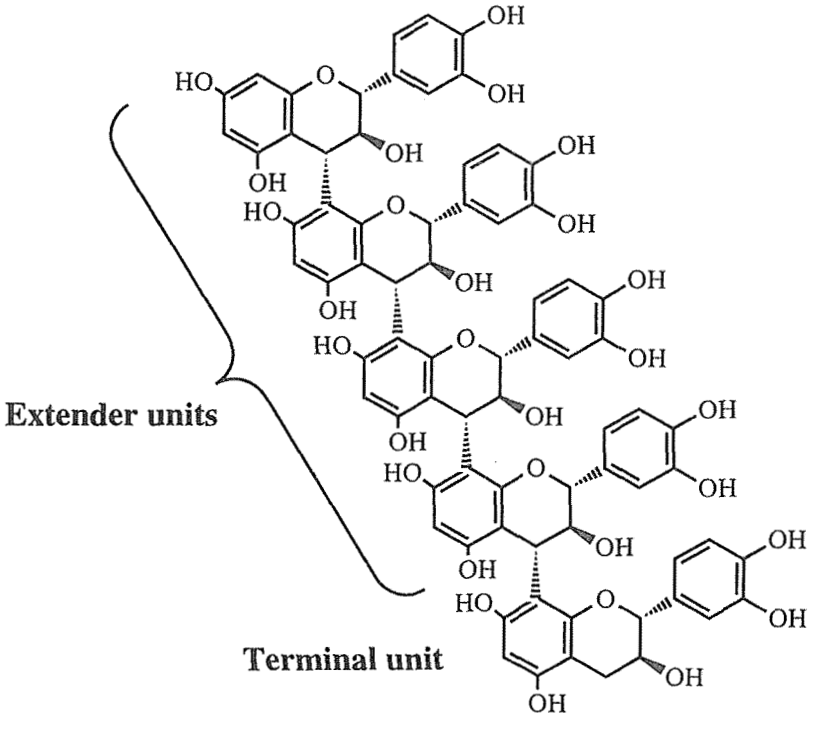
In spite of their special properties such as electrophilicity, metal chelation, and protein



Flavan-3-ol
[(+)-catechin]



Procyanidin dimer



Procyanidin oligomer

precipitation which cannot be found in other components of wood, the bark containing a large quantity of condensed tannins are utilized merely as a fuel, fertilizer, or bedding for livestocks in Japan.[27] In South Africa and United States, on the other hand, the wood adhesives utilizing condensed tannins have been examined by several researchers[28-30] since the 1970's. However, condensed tannins are used merely for the replacement of the expensive phenols such as resorcinol or phloroglucinol in those utilizations, and it is difficult to say that they contribute to the capabilities of the adhesives.

Because of the helical conformations of oligomeric procyanidins, as mentioned by Haslam,[31] it is presumed that the A-ring and heterocyclic ring are distributed to the internal site, and the B-ring is done to the outer site in their molecules. These structures

are not preferable considering the reactivities on the A-ring. Therefore, the structural modification for increasing the flexibility of their molecule should be needed to create the epoch-making utilities. The author gave attention to the pyran-ring opening reaction as one of the structural modification in this thesis.

Sulphitation or metabisulphitation which is one of the oldest, but potentially most useful reactions in the preparation of adhesives based on condensed tannins had been believed as the reaction accompanying the pyran-ring opening.[32] In a investigation later, however, a pyran-ring opened compound from (+)-catechin was isolated in very low yield (*ca.* 1%), and was identified as sodium 1-(3,4-dihydroxyphenyl)-2-hydroxy-3-(2,4,6-trihydroxyphenyl)-propane-1-sulphonate by Foo et al.[33] Therefore, they concluded that the marked improvement in water solubility of tannin isolates and the reduction in solution viscosity resulting from sulphonation of condensed tannins can be explained by cleavage of the interflavanoid bonds not by opening of the pyran-ring.

In this thesis, the author discussed the mechanisms of the pyran-ring opening reaction of (+)-catechin or natural condensed tannins in the presence of boron trifluoride (BF_3) and excess of phenol for the reforming of condensed tannins.

In chapter I, the phenolation behavior of (+)-catechin in the BF_3-phenol system was described and chemical structure of several phenol adducts were identified. Additionally, the influences of solvents and catalysts on the formation of a pyran-ring opened compound (Compound I) were discussed.

In chapter II, an analytical method, methylation-Nucleus Exchange Reaction (M-NER), was developed to determine the opening unit of pyran-ring.

In chapter III, the characteristics of the phenolation products obtained from bark extracts were discussed from viewpoints of the reactivities with formaldehyde and the inhibitory activities for glucosyltransferase (GTase) relating to the generation of dental caries.

Chapter I
Phenolation mechanisms of (+)-catechin in the presence of BF_3

I-1 Phenolation behavior and the identification of the phenolation products

I-1-1 Introduction

Condensed tannins are widely distributed in the plant kingdom, and it is reported that their contents reach more than 10% based on the bark in western hemlock.[34]

The chemical structure of condensed tannins are predominantly procyanidin polymers consisting of chains of polyhydroxyflavan-3-ol unit linked through C4-C6 or C4-C8 bonds.[11,35-38] In applied studies, they have been used as wood adhesives,[28,29,39] for leather tanning,[40] and mud additives[34] because of their polyphenolic properties.

Chemical modification of condensed tannins have been studied by Pizzi[41] and Roux[42] to develop applied research for cold-setting wood-laminating adhesives. Hemingway and Kreibich[43] also demonstrated the presence of oligomeric procyanidin-4-resorcinol adducts in the reaction mixtures when extractives of loblolly pine (*Pinus taeda* L) was treated with resorcinol under acidic conditions. Furthermore, sulfonation[32] of condensed tannins had been thought to improve water solubility and reduce solution viscosity by opening the pyran-ring. However, Foo and co-workers demonstrated that the 4-sulfonated adducts and little pyran-ring opened compound were formed in the sodium hydrogen sulfite treatment of loblolly pine.[33] Sasaya and others[44] expected the formation of a pyran-ring opened and resorcinol adduct compound from taxifolin during solvolysis at 180℃ for 12 hrs, but benzyl coumaranone derivatives was identified as the main product. Recently, in an alkaline condition, a pyran-ring opened and phloroglucinol-adduct compound was identified from procyanidin dimer via a quinone methide intermediate.[45] But, this compound was unstable under alkaline conditions, as it changed into more complex compound, such as catechinic acid which would be less reactive to nucleophilic reagents than would pyran-ring opened products. Therefore, the method for obtaining of the pyran-ring opening products was proposed here for increasing the rotational freedom of A-ring of condensed tannins. Abe and Funaoka indicated the phenolic patterns of A- and B-rings of several condensed tannins from quantities of liberated phenols which could be produced via pyran-ring opened intermediates by running the Nucleus Exchange Reaction.[46]

In this chapter, the phenolation of (+)-catechin, one of the monomeric unit of condensed tannins, by using an excess of phenol in the presence of BF_3 which is known as a catalyzer of cationic ring opening polymerization was attempted. Additionally, the

chemical structures of phenol adducts produced in the phenolation were identified by means of a high resolution nuclear magnetic resonance.

I-1-2 Experimental

Phenolation

(+)-Catechin (Nacalai Tesque Inc. Kyoto Japan) was recrystallized from hot water. The catalyst used was BF_3-phenol complex (Nacalai Tesque Inc. Kyoto Japan). Xylene and phenol (Wako Pure Chemical Industries. Ltd.) were used as diluent and solvent, respectively. Reaction was carried out in a 10ml glass ampoule with 50mg of (+)-catechin and 3ml of the reagent at a temperature of 40-60℃. The constitution of the reagent was xylene : phenol : BF_3-phenol complex=10 : 19 : 1 (v/v). After the reaction had been finished, the reaction mixture was poured into an excess of saturated brine and extracted with ethyl acetate (30ml x 3). After having been dried over sodium sulfate, the solution was evaporated up to a small volume (4-5ml), and then was added dropwise into benzene (100ml) to remove excess phenol. Brownish precipitates were collected by centrifuging at 4000rpm (2000g) and dried in vacuo over P_3O_5.

General analyses

Analytical separation by TLC (thin layer chromatography) were performed on Merck 60F254 silica-gel precoated plates (developing solvent A; toluene : ethyl acetate : acetic acid=5 : 4 : 1, solvent B; benzene : ethyl acetate=4 : 3). Spots were visualized on silica gel plates by vanillin-HCl spray and UV (ultraviolet) illumination. The quantitative analyses of liberated catechol in the benzene soluble fraction and of Compound I, II, and III in the precipitates were made by means of HPLC (high performance liquid chromatography) with an internal standard method by using saligenin. A Jasco 800series system (column: Develosil ODS-10, 4.6mmϕ x 250mm; mobile phase: MeOH/0.01% trifluoroacetic acid=28/72 — 45/55 convex gradient for 40min; Flow rate: 1.0ml/min; Detector: UV 280nm) was used for the HPLC.

Isolation and identification of Compound I, II, and III

Because the precipitates mainly consisted of compounds I, II, and III judging from the HPLC, these compounds were separated by preparative HPLC (column: Develosil ODS-10, 20mmf x 250mm; mobile phase: MeOH/0.01% TFA=30/70 for Compound I, 40/60 for compounds II and III; Flow rate: 10.0ml/min; Detector: UV 280nm). The UV(ultraviolet) spectrum was measured in methanol on a Jasco UVIDEC-505 spectrophotometer. Mass spectrum was measured by SIMS (secondary ion mass spectroscopy), FDMS (field desorption mass spectroscopy), and EIMS (electron impact mass spectroscopy) on a Hitachi A-80. ^1H- and ^{13}C-NMR (nuclear magnetic resonance) spectra were measured on a JNM-GSX 400

spectrometer. The following NMR pulse sequences were used: ^1H-^1H COSY (Correlation spectroscopy) was recorded using a flip angle of 90°. ^1H-^1H NOESY (nuclear Overhauser effect and exchange spectroscopy) was recorded using a mixing time of 750ms. The long-range ^1H-^1H COSY experiment used a 90° mixing pulse and a delay time of 350ms.

Compound I

Rf (solvent A): 0.37, UV λ_{max}^{MeOH} nm (log ε): 279 (3.71), SIMS m/z: 385(MH^+), 355, 324, 281, 251, 206, and 132 (Glycerol was used as a matrix.). ^1H-NMR (1% in $CDCl_3$): δ 2.25 (1H, doublet(d), J=10Hz), 2.42 (1H, d, J=10Hz), 3.78 (1H, d, J=8Hz), 4.29-4.48 (1H, multiplet (m)), 5.93 (2H, singlet (s)), 6.73 (2H, d, J=8.5Hz), 6.77 (1H, d, J=11.5Hz), 6.78 (1H, d, J=11.5Hz), 6.78 (1H, s), and 7.25 (2H, d, J=8.5Hz).

Acetate of Compound I (I-Ac)

About 10mg of Compound I was acetylated with acetic anhydride (200 μl) and pyridine (200 μl) at the ambient temperature for 24 hours. I-Ac was purified on the preparative TLC (developing solvent: benzene/ethyl acetate=3/2).

Rf (solvent B): 0.50, EIMS m/z: 618 ($M^+ - CH_3COOH$), 341, 299, 277, 257, 235, 215, 193, and 151. ^1H-NMR (1% in $CDCl_3$): δ1.756 (3H, s), 2.145 (3H, s), 2.242-2.281 (15H, s), 2.758 (1H, double doublet (dd), J=14Hz, 5.5Hz), 2.843 (1H, dd, J=14Hz, 8.4=Hz), 4.122 (1H, d, J=7.0Hz), 5.759-5.776 (1H, m), 6.855 (2H, s), 7,014 (1H, d, J=8.0Hz), 7.039 (2H, dd, J=8.4Hz, 2.0 Hz), 7.106 (1H, d, J=1.6Hz), 7.118 (1H, dd, J=8.0Hz, 1.6Hz), 7.339 (2H, dd, J=8.4Hz, 2.0 Hz).

Compound II

Rf (solvent A): 0.57, UV nm (log ε): 280 (3.86) (no maximum between 200nm and 400nm), SIMS m/z: 383 (MH^+), 367, 350, 337, 206, and 182. (glycerol was used as a matrix.)

Methyl ether of Compound II (II-Me)

Compound II (20mg) was dissolved in 15ml of acetone, and 0.25ml of dimethyl sulfate and 1.5g of potassium carbonate were added to the acetone solution. Then it was refluxed for 17 hours. The main compound detected by HPLC from the reaction mixture was collected with preparative HPLC, eluent: MeOH / 0.01% trifluoroacetic acid (TFA), column: ODS-20φ x 250mm, flow rate: 10ml / min. Then, 16.5mg of Compound II-Me was obtained as white amorphous by freeze drying.

^1H-NMR (1% in $CDCl_3$): d2.32 (1 H, dd, J=14.4 Hz, 4.4 Hz), 3.401 (1 H, d, J=14.4 Hz), 3.685 (1 H, d, J=9.6 Hz), 4.774 (1 H, quadruple doublet, J=9.6 Hz, 4.4 Hz, 1.6 Hz), 3.644 (3H, s), 3.822 (3H, s), 3.883 (3H, s), 3.900 (3H, s), 3.903 (3H, s), 6.390 (1H,

s), 6.548 (1H, d, J=2.4 Hz), 6.652 (1 H, d, J=2.4 Hz), 6.927 (2 H, d, J=8.4 Hz), 6.963 (1 H, s), 7.254 (2 H, d, J=8.4 Hz).

^{13}C-NMR (1% in $CDCl_3$) : δ30.746 (C-7), 53.754 (C-5), 55.229 (4′-OMe), 55.539 (10-OMe), 55.777 (3-OMe), 56.059 (8-OMe), 56.101 (2-OMe), 79.839 (C-6), 97.309 (C-9), 105.095 (C-11), 111.054 (C-4), 111.954 (C-1), 114.189 (C-3′, 5′), 117.070 (C-7a), 130.675 (C-2′, 6′), 131.434 (C-1′), 132.418 (C-12a), 132.966 (C-4a), 142.720 (C-11a), 147.456 (C-2), 147.920 (C-3), 158.377 (C-8), 158.545 (C-4′), and 159.248 (C-10).

Compound III

Rf (solvent A): 0.65, UV λ_{max}^{MeOH} nm (log ε): 279 (3.62), SIMS m/z: 367 (MH^+), 355, 343, 337, 325, 315, 223, 199, and 123. ^{1}H-NMR (1% in $CDCl_3$) : δ2.65 (1 H, dd, J=15.0Hz, 7.0Hz), 2.93(1 H, m), 5.69 (1 H, d, J=2.0Hz), 5.80 (1 H, d, J=2.0Hz), 6.68 (2H, d, J=10Hz), 6.65-6.80 (3H, m), and 7.15 (2 H, d, J=10.0Hz).

Acetate of Compound III (III-Ac)

About 10mg of Compound III was acetylated with acetic anhydride (200ml) and pyridine (200ml) at the ambient temperature for 24 hours. III-Ac was purified on the preparative TLC (developed with solvent B).

Rf (solvent B): 0.65, EIMS m/z: 576 (M^+), 342, 299, 257, 234, 215, 151, and 123. ^{1}H-NMR (1% in $CDCl_3$) : d2.2-2.3 (15H, s), 2.82 (1H, dd, J=15.7Hz, 7.7Hz), 3.10 (1H, dd, J=15.7Hz, 8.8Hz), 4.20 (1H, d, J=9.2Hz), 5.44-5.53 (1H, m), 6.39 (2H, s), 7.04 (2H, d, J=8.8Hz), 7.08-7.15 (3H, m), and 7.30 (2H, d, J=8.8Hz).

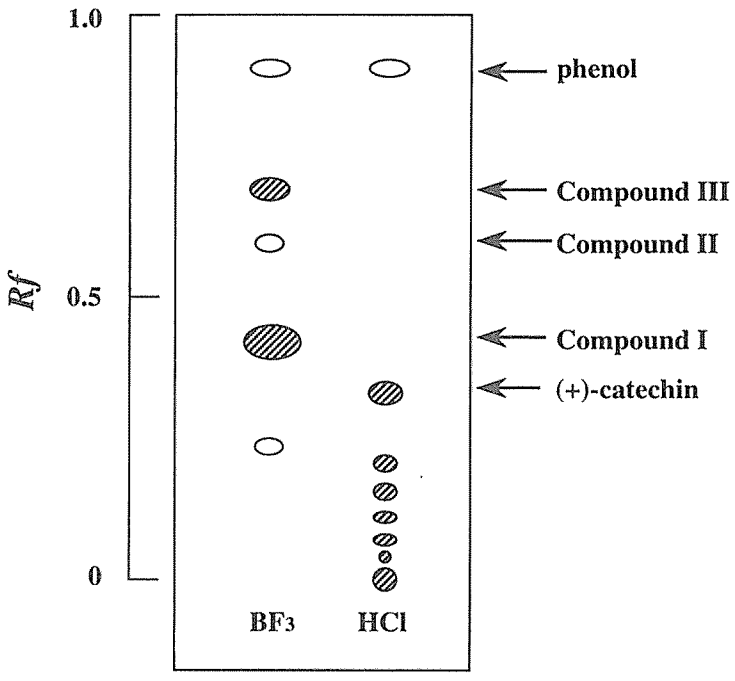


Fig.1 TLCs of phenolation products from (+)-catechin.

Legend: ▨ : vanillin-HCl positive spots.

Note: Developing solvents used was toluene; EtOAc : AcOH = 5 : 4 : 1.

I-1-3 Results and Discussion

Catalytic activity of BF_3

Catalytic functions of BF_3 are similar to the types of Friedel-Crafts catalysts. They have the following remarkable features, small atomic nuclei, gaseous and high electrop hilicity. Moreover, since BF_3 has the coordinating ability as well as

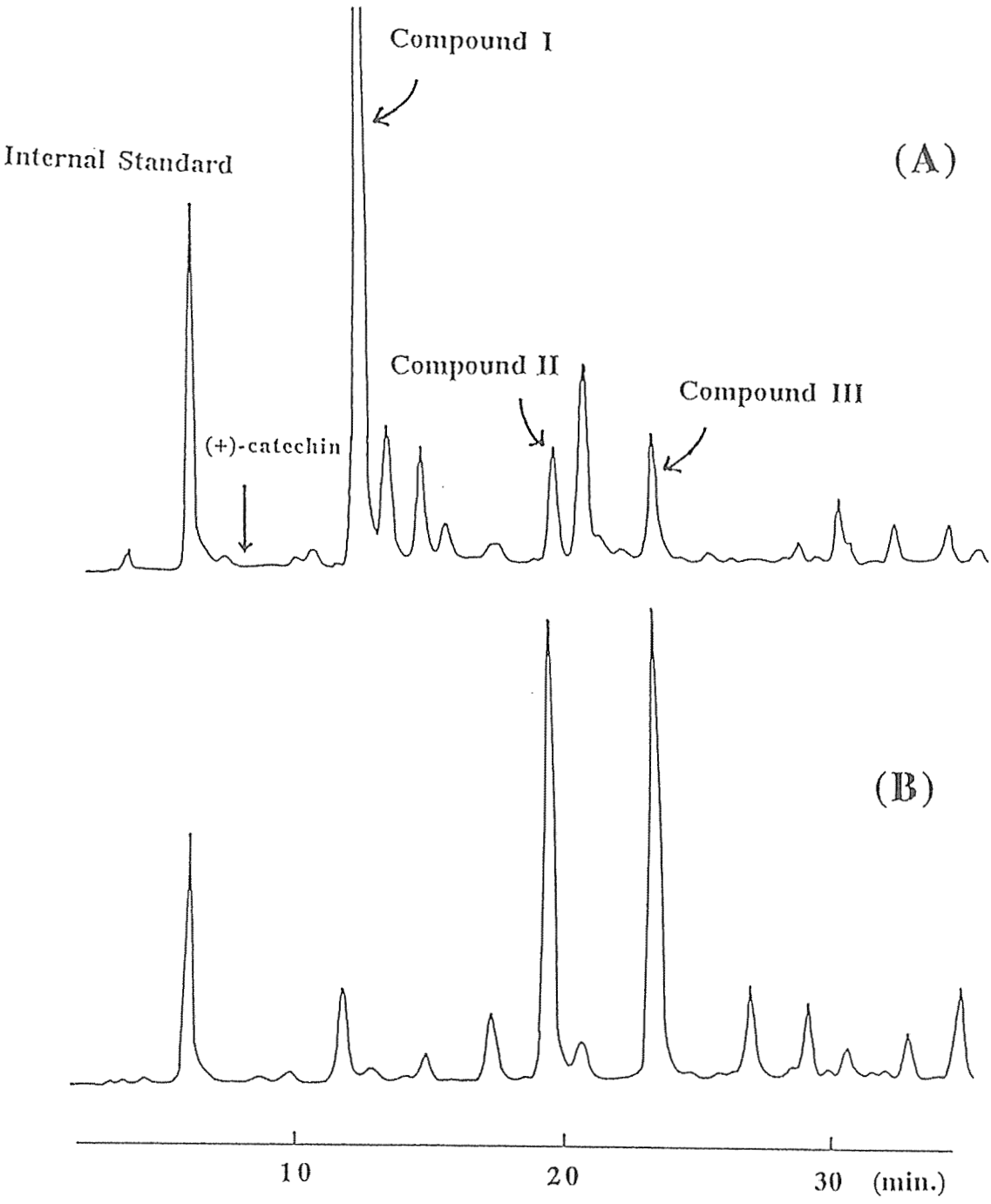


Fig. 2 HPLCs of phenolation products of (+)-catechin with BF_3.

Note: The phenolation of (+)-catechin 50 mg was conducted with the phenolation reagent containing 0.5 mmol BF_3 at 40 °C for 30 min (A) and 240 min (B).

other Lewis-acids do, chemical reaction such as alkylation, polymerization, acylation, and substitution are accelerated with minor side-reactions.

Therefore, the phenolation by using BF_3 was attempted for the phenolation of (+)-catechin. Fig.1 shows TLCs of reaction products from (+)-catechin in the phenolation by the use of 0.5mmol of BF_3 or hydrochloric acid at 40℃ for 30min. The presence of many small Rf value spots indicates that complicated reaction took place during the phenolation in the presence of hydrochloric acid which has been examined so far for the phenolation of condensed tannins. On the other hand, only a few phenolation products were detected in spite of consuming most of (+)-catechin on the BF_3-catalyzed phenolation. From the facts presented above, it became apparent that the phenolation in the presence of BF_3 would be appropriate to understand the main reaction of (+)-catechin.

Identification of the phenolated compounds

HPLCs of the phenolation products are shown in Fig.2. Surprisingly, (+)-catechin (retention time (r.t.): 6.5min) disappeared in the step of initial reaction time, and mainly converted into Compound I (r.t.: 12.61), Compound II (r.t.: 19.85), and Compound III (r.t.: 23.58). After that, Compound II and III were produced largely as main products in consistent with the disappering of Compound I. Compound I was isolated from the precipitate obtained by a reaction at 40℃ for 30min with a 40% yield and Compound II and III were isolated from that obtained a reactions at 40℃ for 240min with 25% and 22% yields, respectively, by the preparative HPLC. These compounds were brownish amorphous solids and dissolved easily in several polar solvents. Their acetates were white amorphous solids and Rf values were 0.37, 0.62 and 0.70, respectively, in the solvent B system.

Compound I

^{1}H-NMR spectrum of Compound I showed ABMX type proton signals at δ 2.25 (1H, dd), 2.42 (1H, dd), 3.73 (1H, d) and 4.29-4.48 (1H, m) ppm. In addition to the signals derived from A- and B-rings, signals at δ 6.73 (2H, d) and 7.25 (2H, d) ppm were observed in aromatic region. It shows that Compound I has the structure adding a phenol with para position. A singlet signal of two protons at 5.93 (2H,s) ppm were attributed to 6-H and 8-H on the A-ring, which showed that this two aromatic protons were equivalence magnetically. However, two aromatic protons on the A-ring of (+)-catechin are not equivalence and are appeared as double doublet signals. This result shows that the Compound I has a phloroglucinol ring appeared by pyran-ring opening of (+)-catechin.

^{1}H-NMR spectrum of Compound I acetate (I-Ac) are shown in Fig.3 (whole region) and Fig. 4 (aromatic region). Intense signals show at 1.756 (3H, s) and 2.145-2.281 (18H, s) ppm, which mean one aliphatic acetyl group and six aromatic acetyl groups. Additionally, the ABMX type proton signals appeared at 2.758, 2.843, 4.122, and 5.759-5.776ppm, which

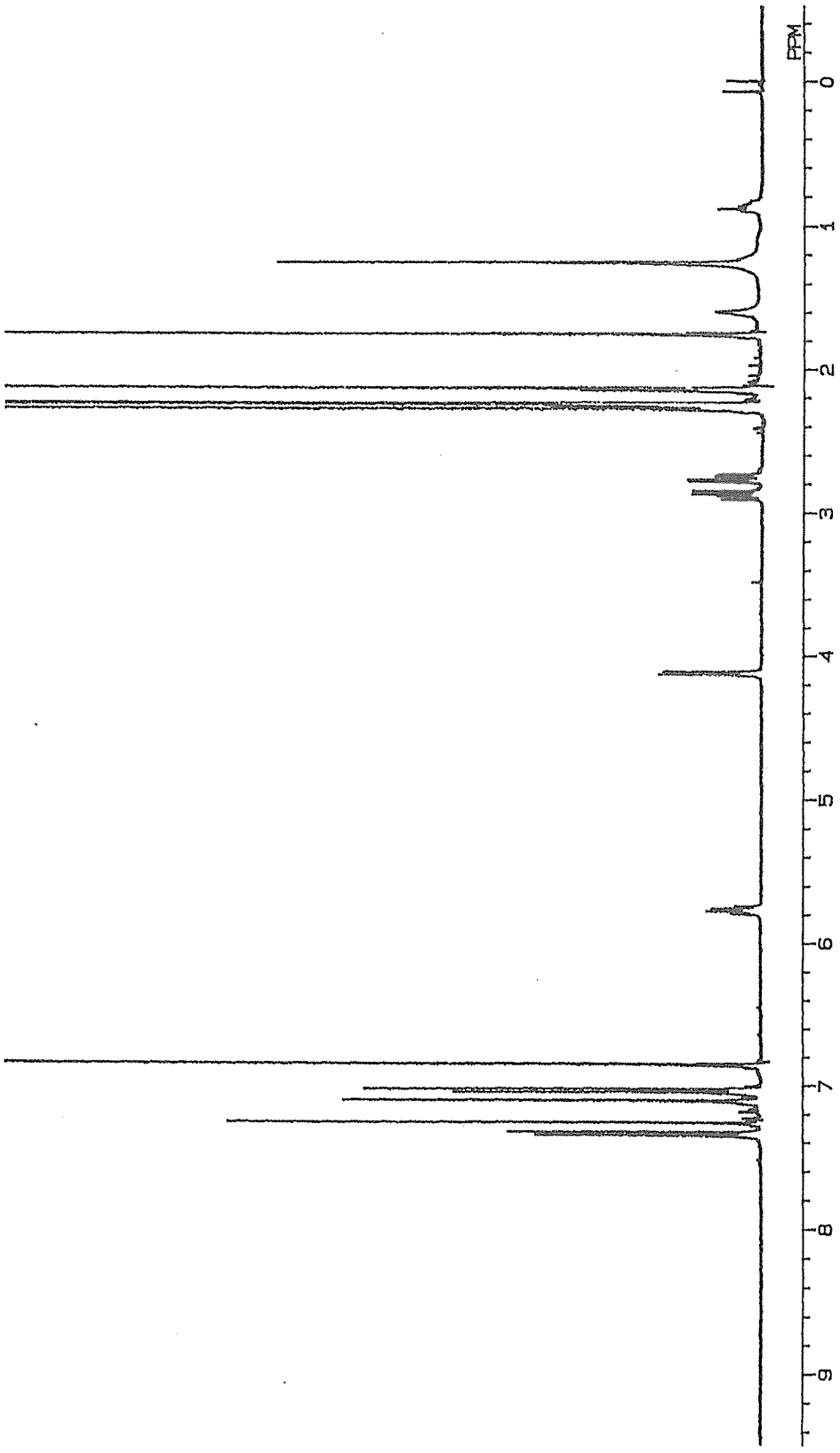


Fig. 3 ^{1}H-NMR spectrum of Compound I-Ac.

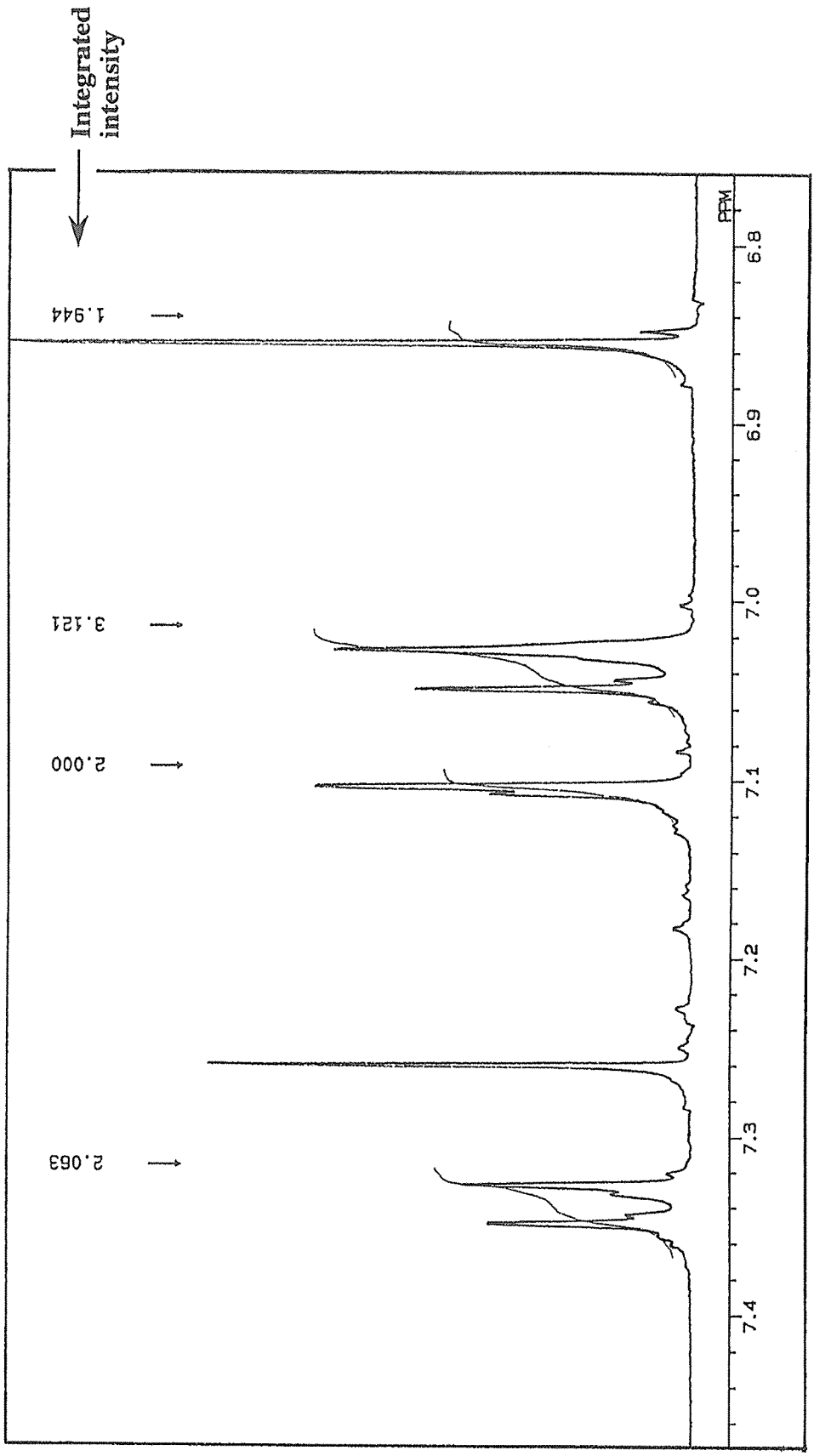


Fig. 4 ^{1}H-NMR spectrum of Compound I-Ac in aromatic region.

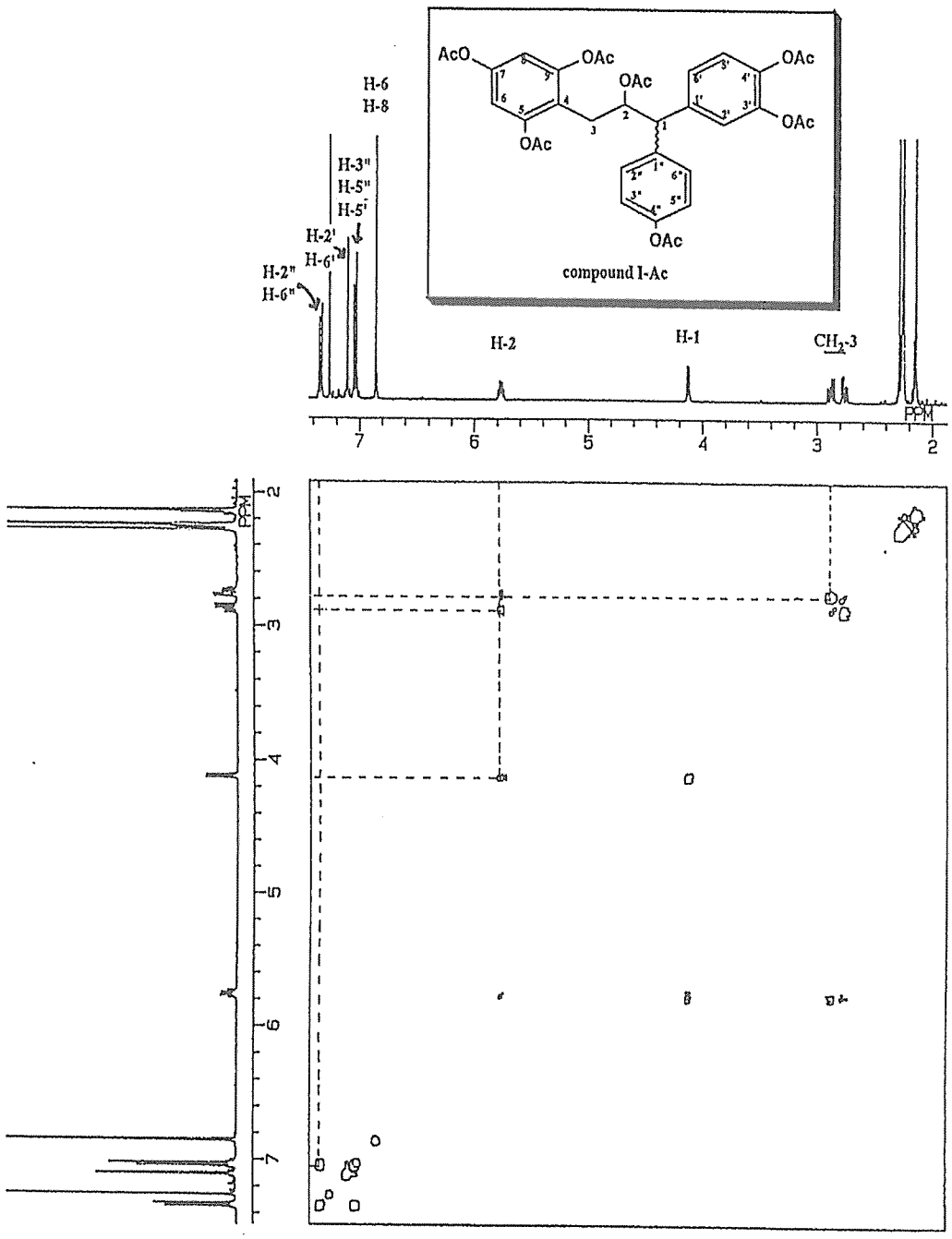


Fig. 5 ^{1}H-^{1}H COSY of Compound I-Ac.

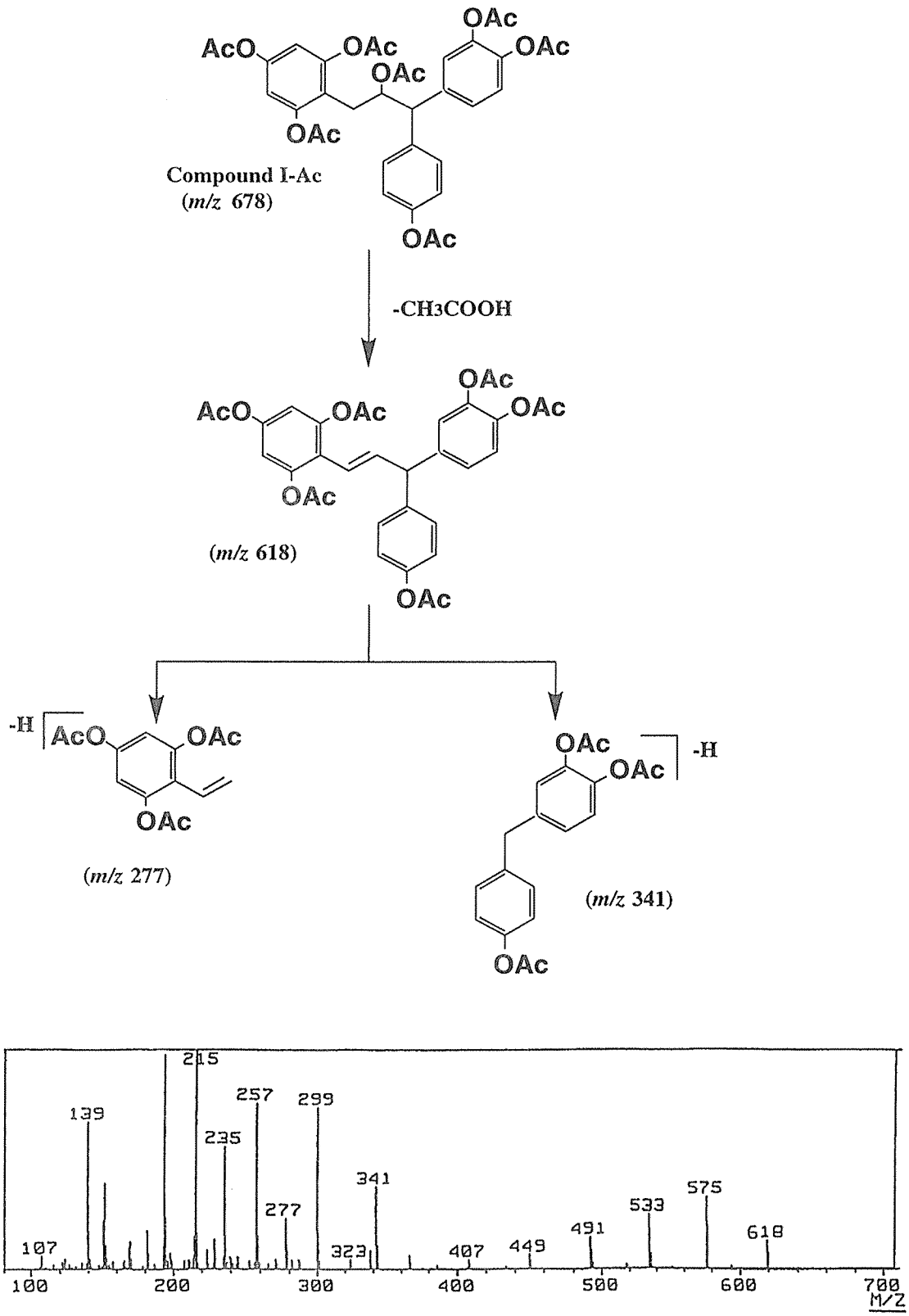


Fig. 6 The major fragmentation pattern of Compound I-Ac on EIMS.

show the presence of a partial structure of $-CH_2-CHCH-$.

In aromatic region (Fig.4), equivalent two protons on A-ring at 6.855ppm and two pairs of ortho-coupled four protons on a phenol ring added at 7.039ppm and 7.339ppm are appeared. ^1H-^1H COSY of Compound I-Ac shown in Fig.5 are consistent with the assignments in ^1H-NMR described above.

The EIMS of I-Ac showed major ions at m/z 618 (M^+-CH_3COOH), 365 (618−6 x CH_2CO), 215 (341−3 x CH_2CO), and 155 (277−3 x CH_2CO). It was thought that m/z 618 was detected as an ion deacetylated from molecular ion m/z 678. Furthermore m/z 341 and 277 were detected as the daughter ions of m/z 618 as shown in Fig.6. Therefore, Compound I was identified as 1-(3,4-dihydroxyphenyl)-1-(4-hydroxy- phenyl)-3-(2,4,6-trihydroxyphenyl) -propan-2-ol, and the configuration of C1 and C2 should be *R* and *S*, respectively based on that of Compound II described later.

Compound II

^1H-NMR spectrum of methylated Compound II (II-Me) was shown in Fig. 7. One double doublet (dd; J=14.4Hz, J=4.4Hz) proton was observed at 2.320ppm (Ha), and one doublet (d; J=14.4Hz) proton at 3.401ppm (Hb). These protons were presumed to be geminal protons judging from the coupling constants. Additionally, one doublet (d; J=9.6Hz) proton and one quadruple doublet (qd; J=9.6Hz, J=4.4Hz, J=1.6Hz) proton were shown at 3.685ppm (Hc) and at 4.774 ppm (Hd), respectively. Some correlations were observed in the ^1H-^1H COSY of Compound II-Me (Fig. 8). The Hd correlated with the Ha, the Hb, and the Hc, and also the Hb correlated with the Ha, which indicated the presence of (−CH_2(a,b)−CH(d)−CH(c)−).

Five singlets signals observed at 3.63ppm~3.91ppm were assigned to methyl protons of five methoxyl groups. Therefore, it was presumed that five phenolic hydroxyl groups existed in Compound II.

In the aromatic region, two doublet (dd; J=2.4Hz) signals correlating with each other appeared at 6.548ppm (Hf) and at 6.652ppm (Hg), and they were assigned to two protons on the A-ring. Because these two protons were not equivalent and had coupling constants corresponding to meta coupling in the same manner as was observed in case of (+) -catechin,[47] it is indicated that Compound II−Me has a heterocyclic ring. Additionally, two doublet (J=8.4Hz) protons correlating with each other appeared at 6.927ppm (Hh) and at 7.254ppm (Hj). As each doublet consisted of two protons and had coupling constants corresponding to ortho coupling, these signals were assigned to the four protons on an adducted phenol−ring. The remaining two singlet signals appearing at 6.390ppm (He) and at 6.963ppm (Hi) were assigned to the two protons locating in the para-position on the B-ring. This result indicates presumably that the 6′-proton of Compound I, which changed into Compounds II and III as mentioned above, disappeared to form a new heterocyclic

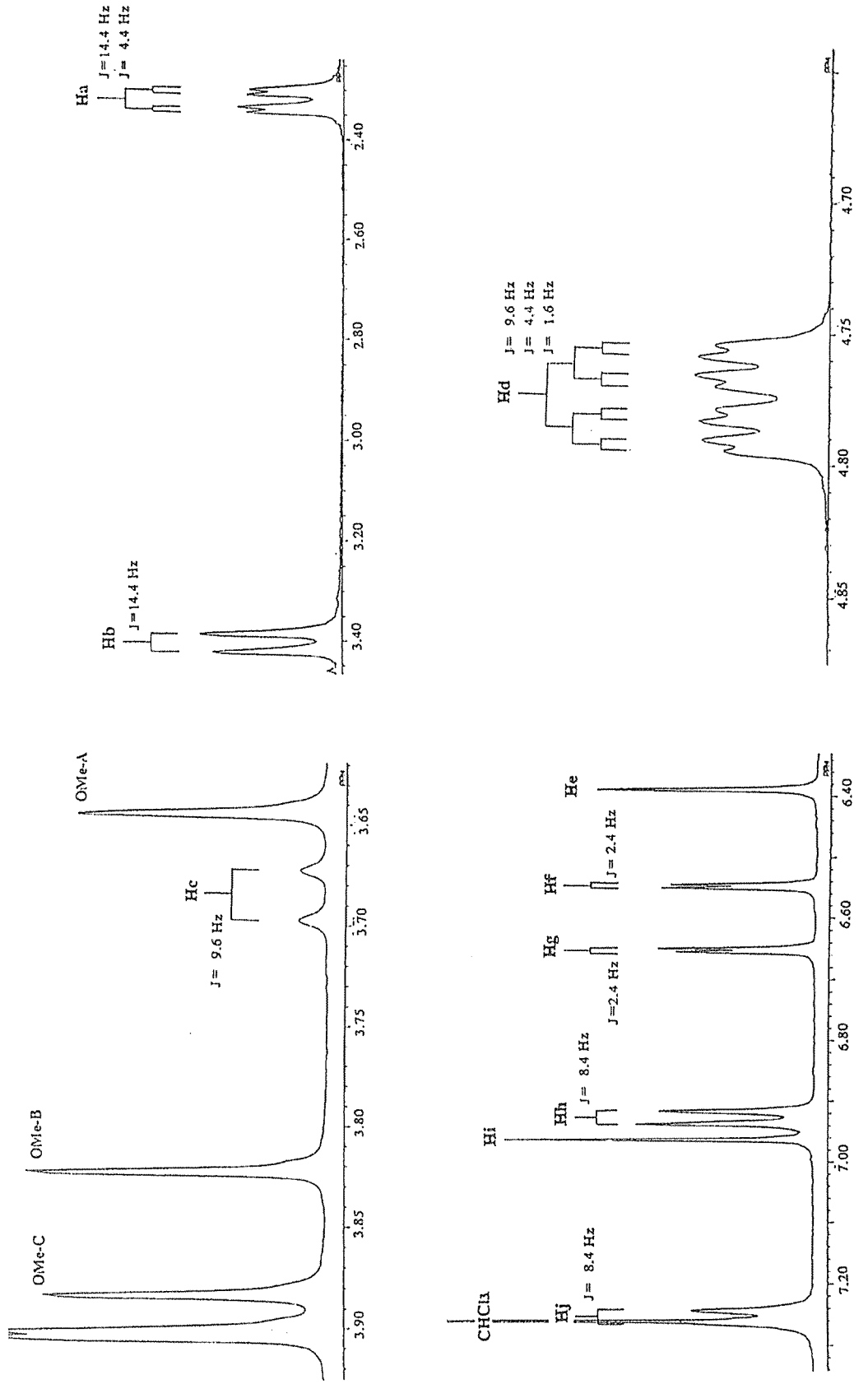


Fig. 7 Expanded charts of ^{1}H-NMR spectrum of Compound II-Me.

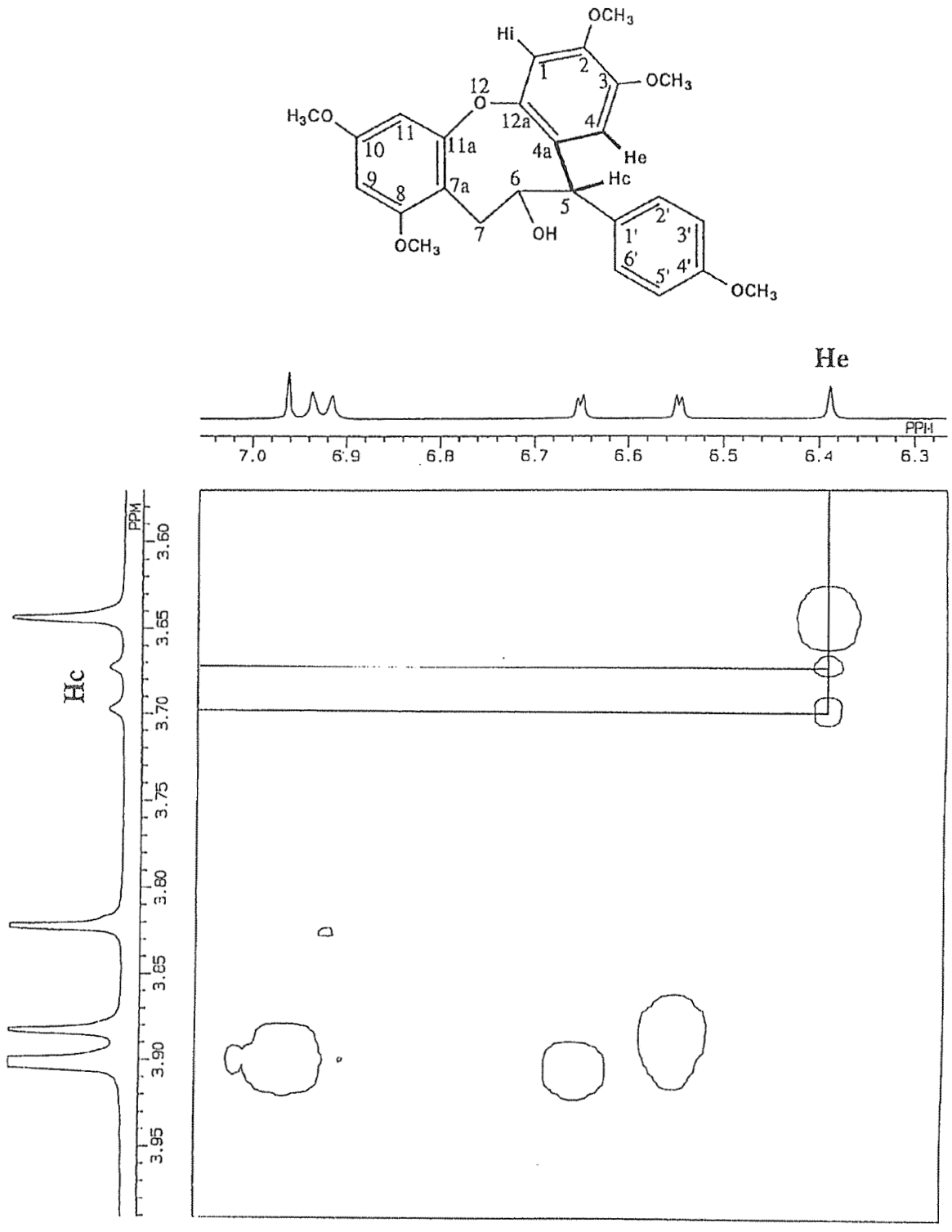


Fig. 8 Long range ^{1}H-^{1}H COSY of Compound II-Me.

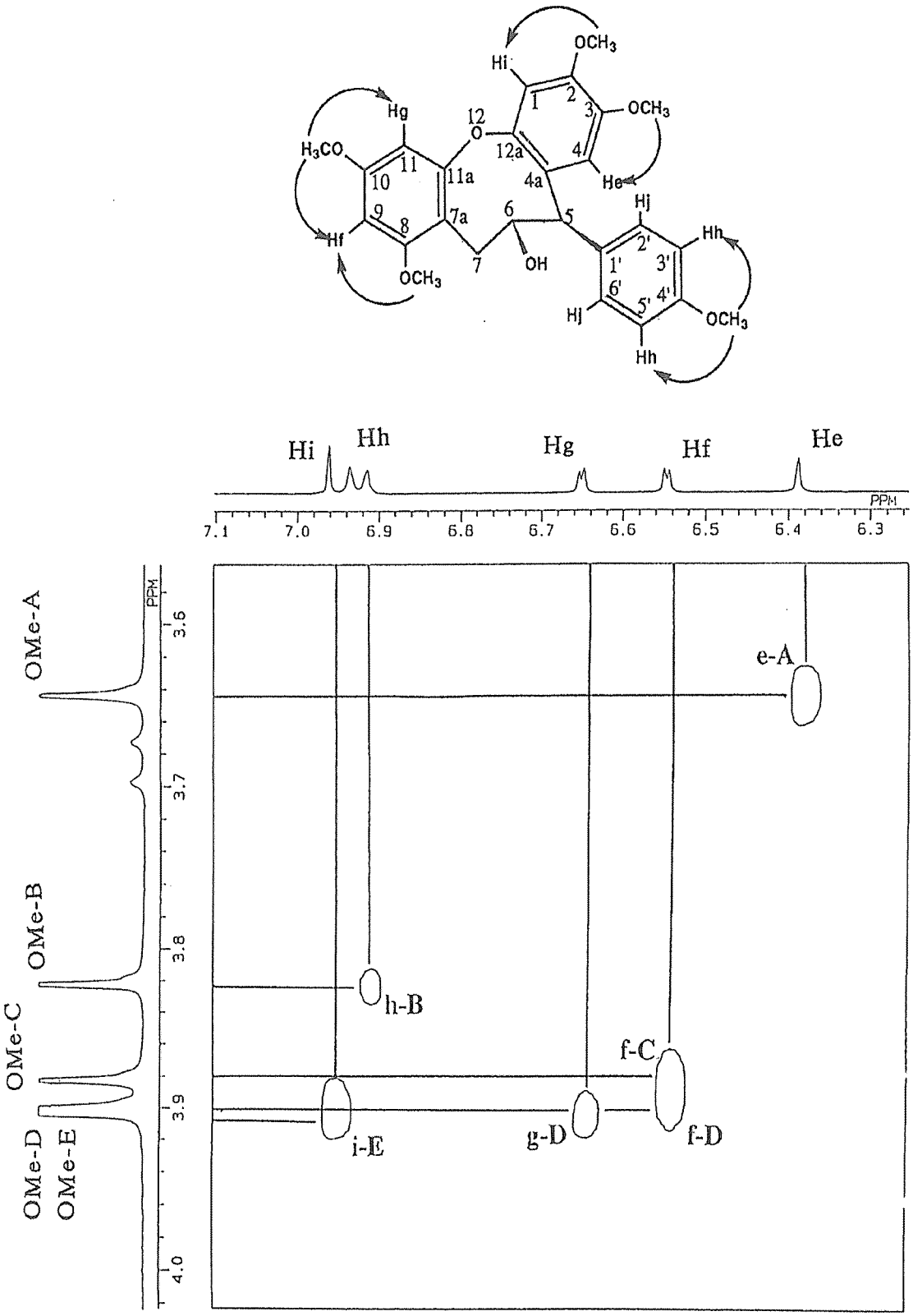


Fig. 9 NOESY of Compound II-Me.

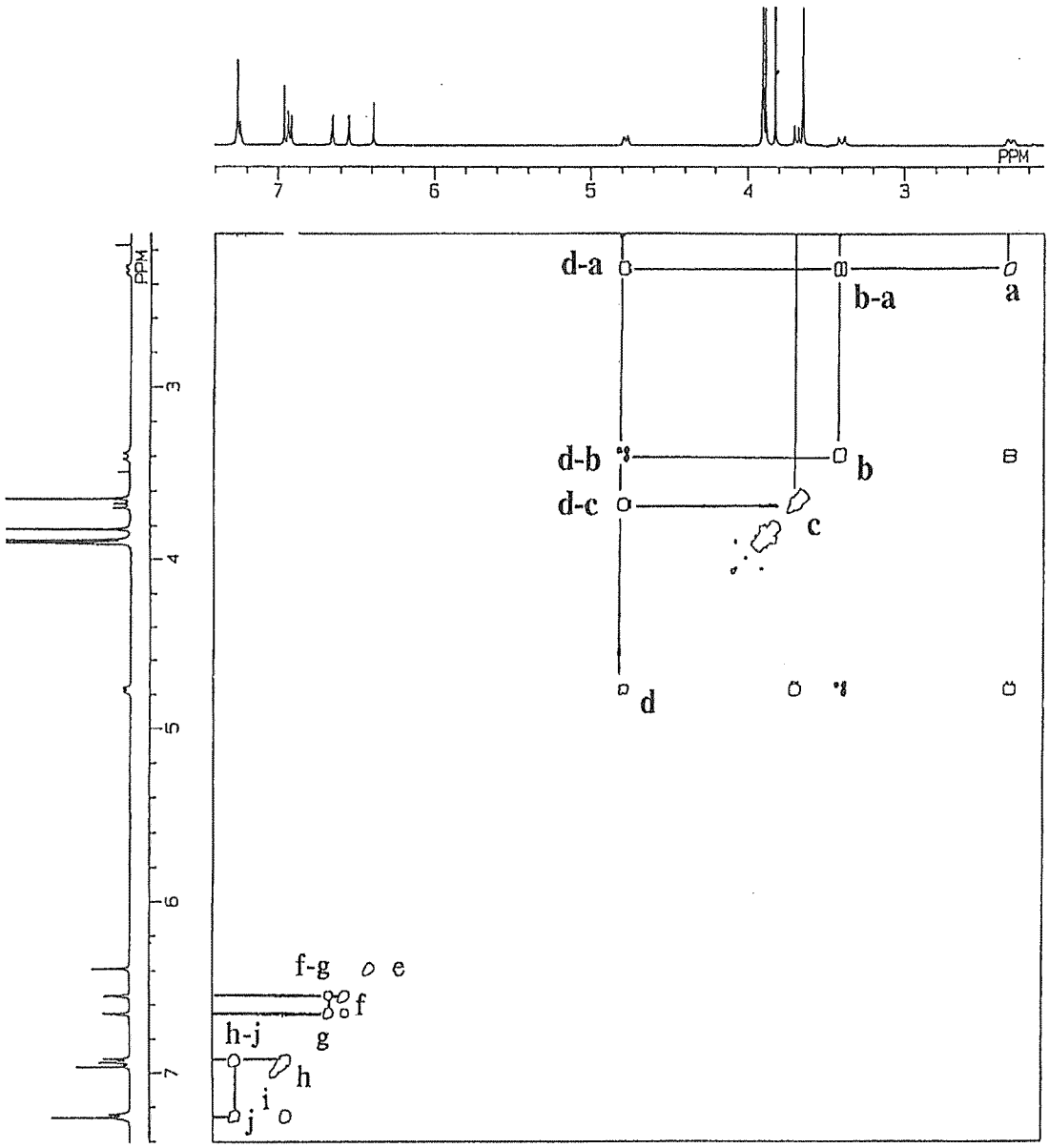
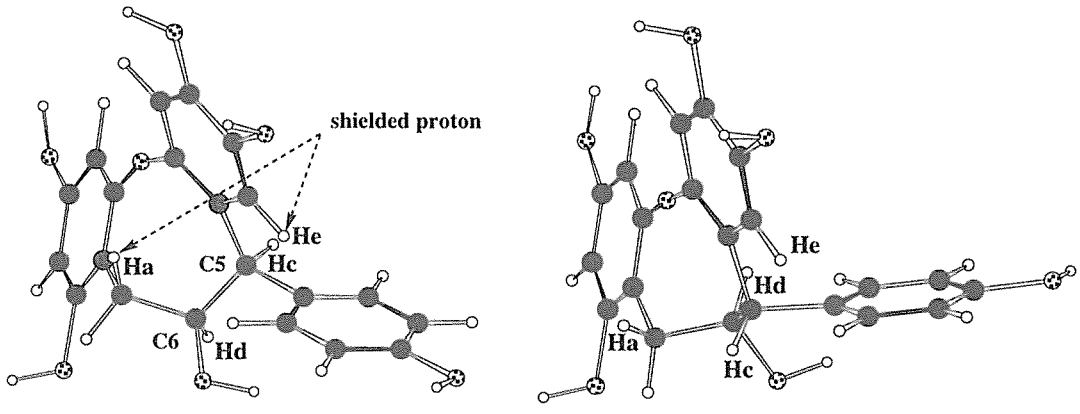


Fig. 10 ^{1}H-^{1}H COSY of Compound II-Me.

ring. Results of NOESY for corelating each aromatic methoxyl group with its neighboring protons were shown in Fig.9. The Hg proton correlated with the OMe−D, and the Hf proton did the same to both the OMe−D and OMe−C, which indicates that the Hg, the Hf, the OMe−D, and the OMe−C are located at the 11−, 9−, 10− and 8−positions on the A−ring, respectively. Because the Hh protons on the phenol ring were correlated to the OMe−B, they are located at 3′- and 5′-positions, and the OMe−B at the 4′−position. The Hj protons that showed no correlation with any methoxyl group should be 2′− and 6′−positions on the phenol ring. As we could not understand the positions of the two

protons on the B−ring, long−range ^1H-^1H COSY was measured as shown in Fig. 10. The Hc proton (5−position) did not correlate with a Hi proton but with a He proton. Furthermore, the He proton appeared at a higher magnetic field than any other aromatic protons in spite of the fact that the B−ring protons emerged normally in a lower magnetic field. This result suggests that the He proton locates in some position which is subjected to a shielding effect from an adducted phenol−ring. Therefore, it has been made clear that the He proton locates at the 4−position and the Hi at the 1−position. Accompanying the above results, it became apparent that the OMe−A and −E located at the 3− and 2−positions, respectively. Fig. 11 shows the stereoviews of two possible configurations at the 5−position and the conformation between the 5- and 6-positions. If the 5−position would be the R configuration and the proton of the 4−position would be located in the shielding field from an adducted phenol−ring as mentioned above, the dihedral angle between the protons of the 5− and 6−positions would be close to 0°. Judging from the relationship between the dihedral angle and the coupling constants,[48] the angle value would correspond to 8∼10Hz. As the observed value of the coupling constant was 9.6Hz as described in Fig. 7, this prediction seems to be reasonable. In this case, it was explicable that the Ha proton also was located in the shielding region from the B-ring because that proton emerged in high magnetic field as shown in Fig. 7. If the 5−position would be S configuration, on the contrary, the dihederal angle of it would be close to 180° judging from that stable comformation. In this case, however, the proton of the 4−position would not be located in the shielding field as shown in Fig. 11. Thus, this prediction seems to be in conflict with the experimental results. Therefore, it was explained that the Compound II would be 5R, 5,6-synperiplanar, and be termed as (5R,6S)-2,3−dihydroxybenzo[b]−8,10−dihydroxybenzo[g]-5-(4-hydroxy-phenyl)-6-hydroxyoxaocane.



5*R*, 5,6-synperiplanar **5*S*, 5,6-antiperiplanar**

Fig. 11 The stereoviews of configuration at C5 and conformation at C5 and C6 of Compound II based on the ^1H-NMR data.

Compound III

The molecular ion peak of Compound III by SIMS showed m/z 366, which was thought to be dehydrated compound from Compound I. ^{1}H-NMR spectrum of Compound III showed ABMX type proton signals at δ 2.65, 2.93ppm (C3-2H), 3.90ppm (Cα-1H), and 5.20~5.55ppm (C2-1H). For aromatic protons, their chemical shifts and coupling constants were very similar to Compound I except for two A-ring protons spliting at 2Hz as observed in the case of (+)-catechin. ^{1}H-NMR spectrum of Compound III acetate (III-Ac) showed that there was five aromatic acetyl groups δ 2.20~2.23ppm and no aliphatic acetyl group as shown in Fig.12. FDMS showed a molecular ion peak at m/z 576, and EIMS showed major ions at m/z 576(M+), 342, 299, 257, 215 (342-3 x CH_3CO), 234, 193, and 151 (234-2 x CH_2CO).

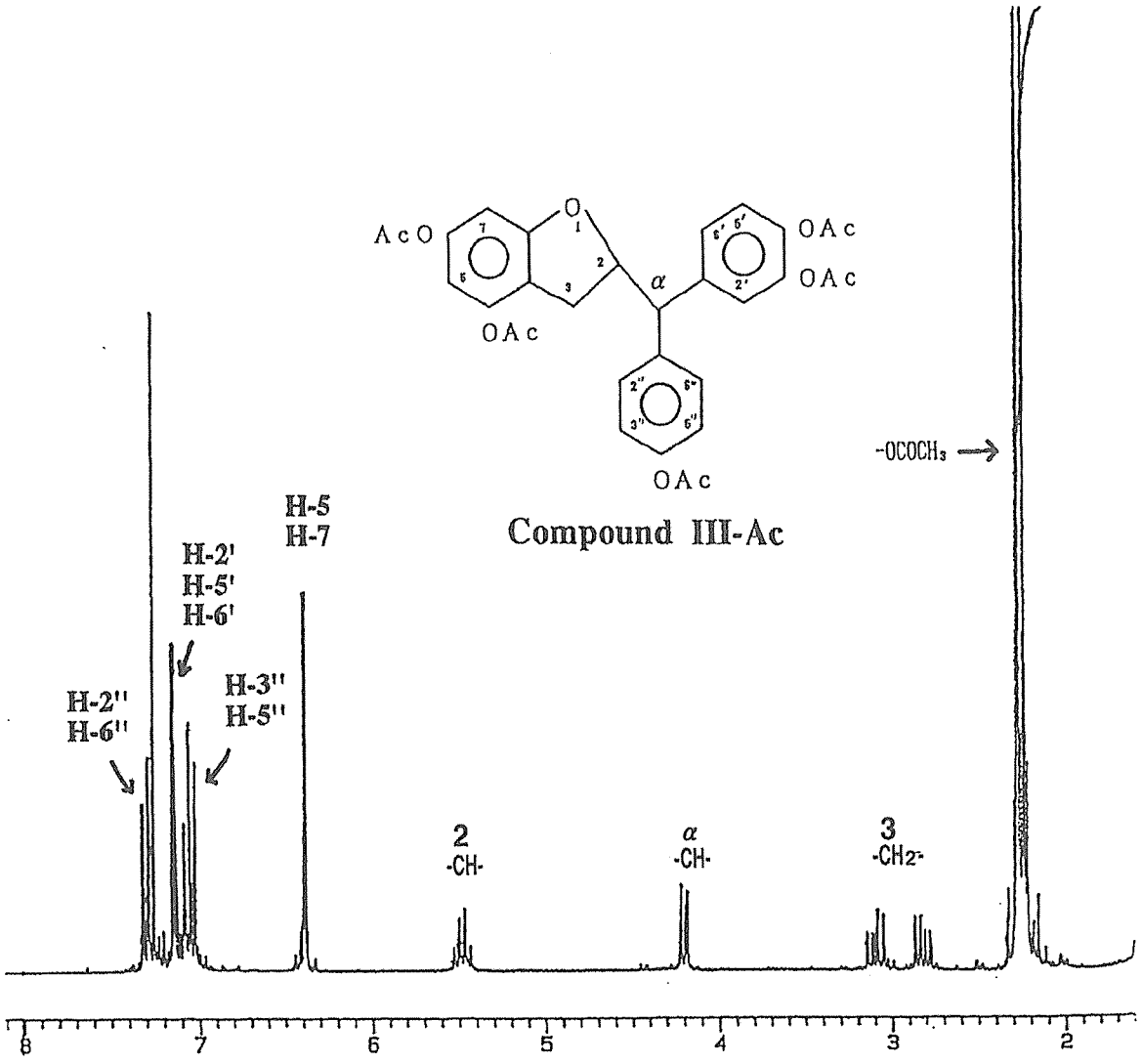


Fig. 12 ^{1}H-NMR spectrum of Compound III-Ac

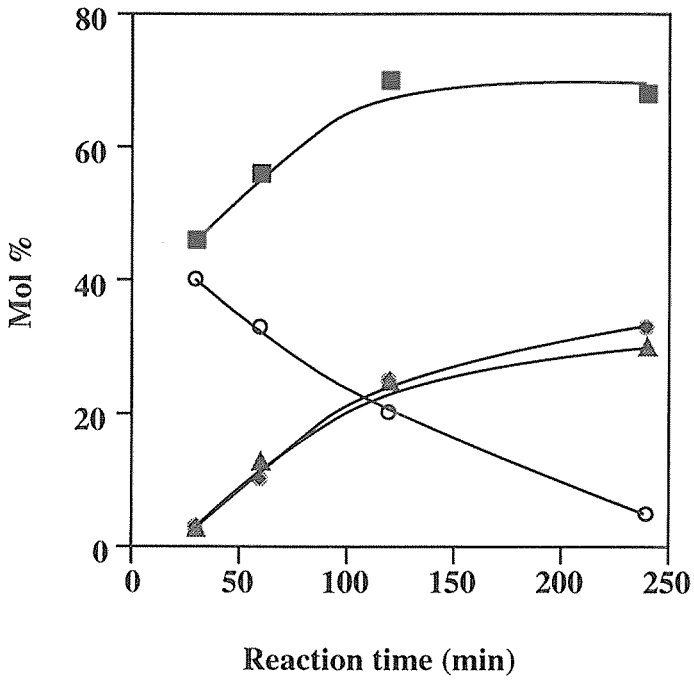


Fig. 13 The yields of Compound I, II, and III from (+)-catechin in phenolation reagent at 40 °C.

Legends: ○ **Compound I** ▲ **Compound II** ◆ **Compound III** ■ **Total**

The ions of 342 and 151 derived from a retro Diels-Alder fragmentation suggested that three acetoxyl groups were attached to the B-ring and a phenyl ring introduced, and two acetoxyl groups were attached to the A-ring. Therefore, Compound III was identified as 4,6-dihydroxy-2-(3,4,4′-trihydroxy diphenyl- methane)-coumaran, and the configuration at C2 and Ca should be *S* and *R*, respectively from that of Compound I.

The formation and mechanism of the phenolation products.

The phenolation was carried out at 40℃ in 3ml of the phenolation reagent because catechol liberation has occurred at 60℃ in the NER reagent as mentioned by Abe et al.[46] Figure 13 shows the changes of the yield of these compounds and their total yiels (mol%) based on (+)-catechin. Compound I linearly decreased with the increase of reaction time, whereas Compound II and III linearly increased up to 120min, and gradually increased after 120min of the reaction time. Furthermore, total yield of Compound I, II, and III reached about 70mol% within 120min. From the fact presented above, it was presumed that the

preferential reaction, in which (+)-catechin changes rapidly into Compound I, and then Compound I gradually transformes into Compound II and III, occurred in this system. The result of quantitative analyses of Compound I, II, III and catechol at various reaction times are shown in Table 1. It is obvious that catechol was not liberated at 40℃, but was gradually produced with rising temperature. The decrease of total yields of these compounds suggests that the side reactions occurred gradually with rising the reaction temperature. The reaction of Compound I in the phenolation reagent was examined to confirm the formation of Compound II and III from Compound I. As shown in Fig.14, the same tendencies as described in the reaction of (+)-catechin were observed that the yields of Compound II and III increased as that of Compound I decreased. Judging from the total yields (90mol%) of these compounds, it is obvious that the conversion of Compound II and III from Compound I are the main reactions at 40℃ in the phenolation reagents.

From the above results, the phenolation mechanism is proposed as shown in Fig. 15. (1) an unshared electron pair of oxygen atom of the pyran-ring coordinates with a BF_3 molecule in an excess of phenol, and then Compound I is produced via the nucleophilic substitution reaction, (2) intramolecular dehydration occurs between an alcoholic hydroxyl group and aromatic hydroxyl group in the Compound I to produce Compound III, on the

Table 1 The yields of compounds produced from(+)-catechin in the phenolation reagent.

[mol %]

Temp-Time (°C-min)	catechol	I	II	III	Total
40- 30	0	38.7	5.6	6.0	50.3
40- 60	0	33.8	12.4	10.9	57.1
40-120	0	20.0	24.1	24.1	68.2
40-240	0.7	6.3	32.1	32.1	70.2
50- 30	0.5	25.3	12.4	29.1	67.3
50- 60	1.4	16.1	16.1	31.8	65.4
50-120	2.5	4.2	13.5	43.6	63.8
50-240	4.1	0	11.4	44.7	60.2
60- 30	3.8	5.2	11.2	46.3	66.5
60- 60	4.6	1.0	10.0	46.3	61.9
60-120	6.1	0	9.8	40.6	56.3
60-240	7.5	0	7.5	37.4	52.4

The yields are represented by mol % based on (+)-catechin.

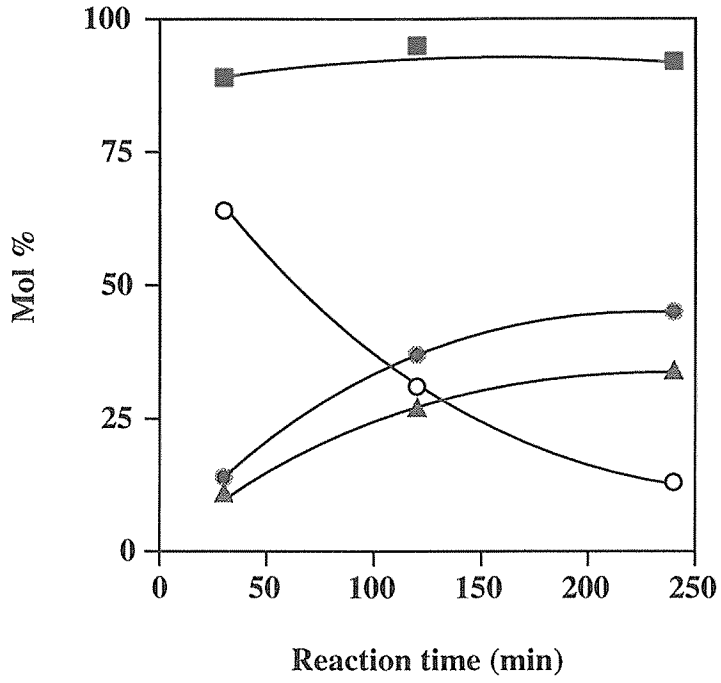


Fig. 14 The yields of Compound II and III from Compound I in phenolation reagent at 40 °C.

Legends: ○ **Compound I** ▲ **Compound II**
● **Compound III** ■ **Total**

other hand, oxidation also occurs to produce Compound II.

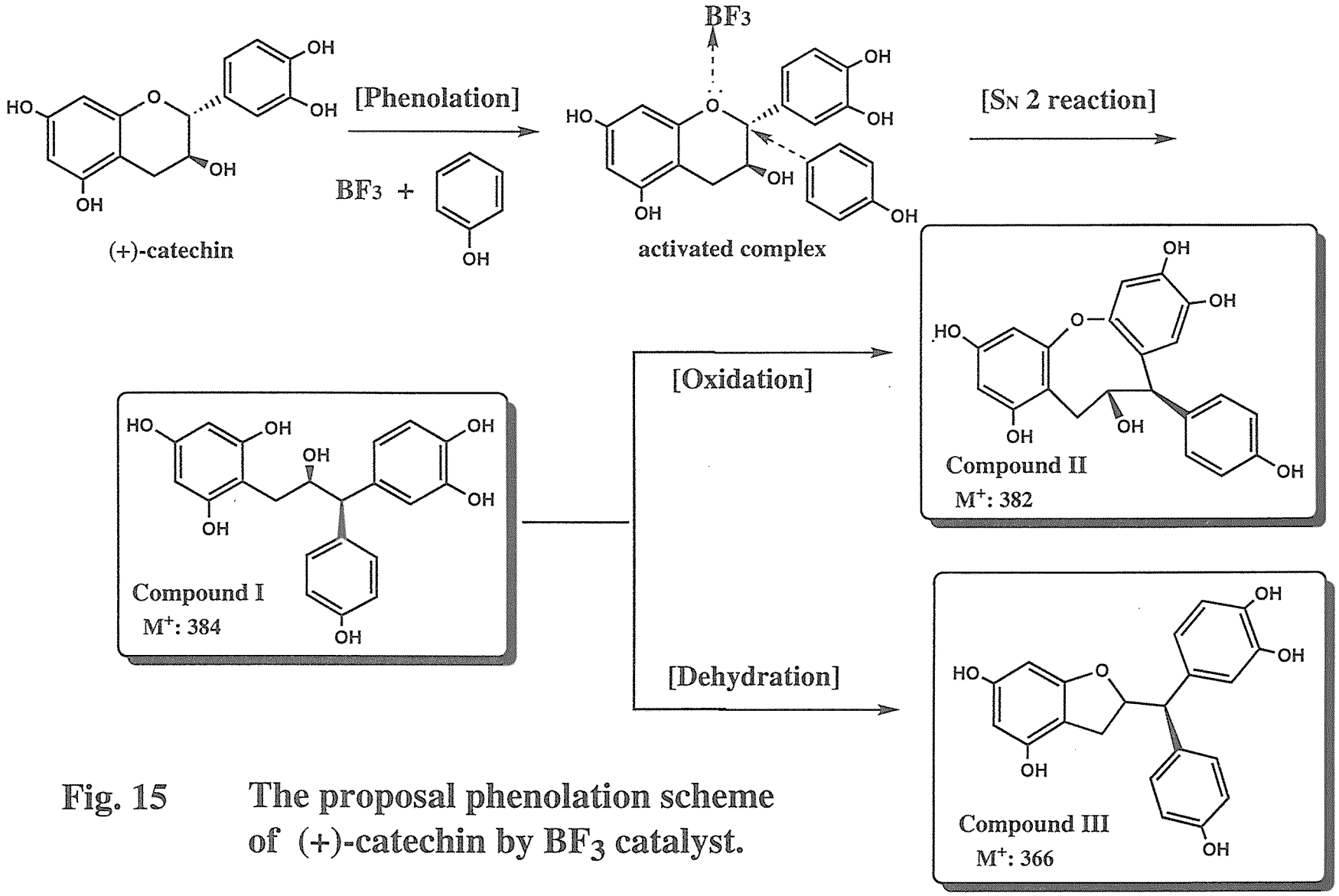


Fig. 15 The proposal phenolation scheme of (+)-catechin by BF_3 catalyst.

I-1-4 Summary

The modification of (+)-catechin by the phenolation was examined in order to understand the phenolation mechanisms of condensed tannins in the pesence of boron trifluoride. (+)-Catechin was transformed into Compound I in the initial stage, and then Compound I changed into Compound II or Compound III during the prolonged reaction. The total maximum yields of these phenolated compounds was 70mol% based on (+)-catechin at 40℃.

Based on the data of ^{1}H-NMR, 2D-NMR, and SIMS Compound I, II, and III were identified as 1-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)-3-(2,4,6-trihydroxy- phenyl)-propan-2-ol, (5*R*, 6*S*)-2,3-dihydroxybenzo[b]-8,10-dihydroxybenzo[g]-5-(4-hydroxyphenyl)-6-hydroxyoxaocane and 4,6-dihydroxy-2-(3,4,4′-trihydroxydiphenylmethane)-coumaran.

Therefore, it was proved that the pyran-ring of (+)-catechin was opened easily by using BF_3 in the presence of excess phenol at 40℃. The phenolation mechanism was presumed that the coordination of BF_3 occurred on the pyran-ring of (+)-catechin, and then nucleophilic substitution took place with a phenol to form Compound I. Furthermore, intramolecular dehydration occurred between an alcoholic hydroxyl group and aromatic hydroxyl group in Compound I to produce Compound III, on the other hand, oxidation also occurred to produce Compound II.

I-2 The influences of the solvents and catalysts on the formation of Compound I

I-2-1 Introduction

Chemical reactions of condensed tannins under acidic and basic conditions predominantly take place at the site of an interflavanoid bond and/or an ether bond on the pyran ring. Hemingway and McGraw,[49] and Hemingway and others[35] pointed out that acid-catalyzed (acetic acid) reaction promoted the interflavanoid bond cleaving and that the C4-C8 interflavanoid bond was cleaved more rapidly than the C4-C6 linked isomers. Furthermore, Hemingway and Daling,[50] and Kreibich and Hemingway[39] reported applicational study for cold setting adhesives. Laks and Hemingway[45] reported a base catalyzed reaction of a procyanidin dimer, in which interflavanoid bond cleaving takes place first, then a pyran ring opens via the formation of quinone methide. Recently, in the treatment of (+)-catechin under mild alkaline conditions (pH 12, 50℃), it changed to the dimeric compound which is the condensation product of catechinic acid and pyran ring opened compound as reported by Ohara and Hemingway.[51]

In chapter I-1, we described the phenolation behaviors of (+)-catechin, which is a monomeric compound of condensed tannins, by a boron trifluoride catalyst and explained the chemical structures of some phenolated compounds. One of them was a pyran-ring opened and phenolated compound (Compound I) which was a main compound in the early reaction step, and it was predicted that the compound was formed via a nucleophilic substitution reaction from (+)-catechin.

In this chapter, the effects of the solvents and catalysts on the formation of Compound I is investigated, and its advanced formation mechanism and stereochemistry are discussed.

I-2-2 Experimental

Phenolation

(+)-Catechin or (+)-epicatechin (50mg) was combined with the reagents containing solvents (1ml), phenol (1.9ml), and catalysts (0.045~4.5mmol) in a glass ampoule. Solvents used were nine aromatic, three protic, and six aprotic solvents as shown in Fig.16, and catalysts were four Lewis acids and five protic acids as shown in Table 2. The reaction was conducted at 40℃ in an incubator after the substrate was dissolved completely with an ultrasonic wave. The reaction mixture was poured into excess saturated brine and was extracted with ethyl acetate (30ml x 3). Then an internal standard compound (saligenin) was added to the ethyl acetate layer. The amount of Compound I was determined by HPLC described in chapter I-1.

Synthesis of (+)-epicatechin

(+)-Epicatechin was synthesized from (+)-catechin by Foo and Porter's method.[52]

(+)-Catechin (500mg) was dissolved in ethanol (3 ml), placed in a stainless-steel bomb, and flushed with nitrogen gas. The reaction was conducted at 180℃ for 2hr in an oil bath. The products were obtained by preparative HPLC, column: Develosil-10, 20mm I.D. x 250mm; mobile phase: MeOH/0.01% TFA=30/70; flow rate: 10ml/min. (+)-Epicatechin (153mg) was obtained as a white amorphous solid after freeze-drying.

Analytical method

Analytical separation by TLC was performed on Merck 60F254 silica-gel precoated plates (solvent; toluene : EtOAc : formic acid=5 : 12 : 1).

^{1}H-NMR spectra of (+)-catechin and (+)-epicatechin were measured in acetone-d_6 on a JEOL EX-270.

I-2-3 Results and Discussion

The effects of the solvents on the formation of Compound I

Since Compound I is a ring-opened and phenolated product of (+)-catechin as identified in chapter I-1, its molecule is expected to be a more flexible structure compared to (+)-catechin. Therefore, it is important for the modification of condensed tannins to find the condition for obtaining a large yield of Compound I. In general, solvent in the reagent is one of the chemical factors influencing the rate of reaction. Figure 16 shows the amount of Compound I after a 30min-treatment at 30℃ when several aromatic, protic, and aprotic solvents were used. (+)-Catechin was almost consumed in most solvents except a few protic solvents. Compound I was formed greatly in the aromatic solvents, especially its yield was 56mol% of (+)-catechin in benzene. It showed more than 2.8times compared with that in xylene which has been used in the NER reagent.[46] As shown in the reaction using xylene, toluene, and benzene, the yield of Compound I increased with a decreasing

Table 2 The several catalysts used for the formation of Compound I.

Lewis acid	Protic acid
BF_3	HF
$TiCl_4$	HCl
$SnCl_4$	H_2SO_4
$AlCl_3$	CH_3COOH
	CCl_3COOH

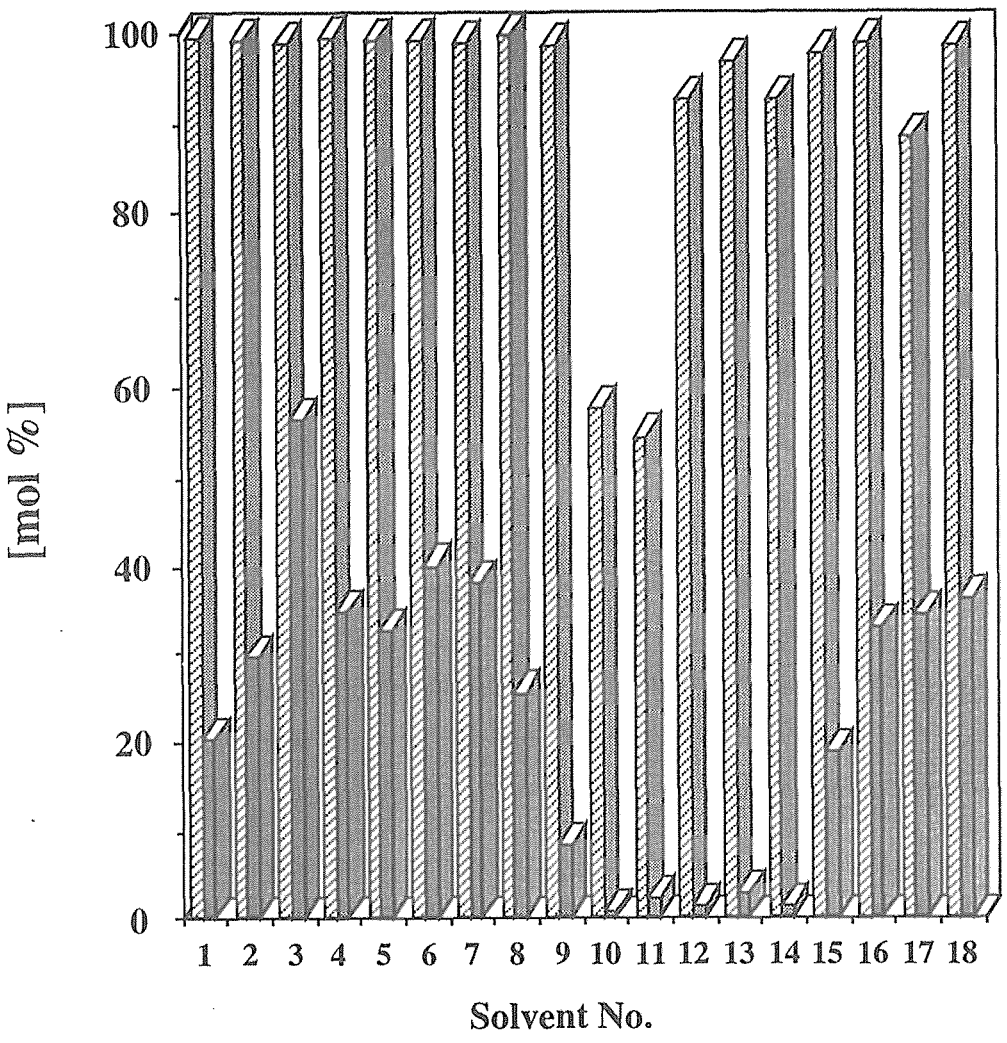


Fig. 16 The effects of several solvents on the formation of Compound I.

Note : Phenolation was conducted at 40 °C for 30 min.

1; xylene, 2; toluene, 3; benzene, 4; ethylbenzene, 5; t-butylbenzene, 6; i-propylbenzene, 7; chlorobenzene, 8; anisole, 9; p-methylanisole, 10; methanol, 11; cyclohexanol, 12; ethylene glycol monomethylether, 13; ethylene glycol dimethylether, 14; dioxane, 15; methyl cyclohexane, 16; dichloromethane, 17; trichloromethane, 18; tetrachloromethane.

Legend : ■ Compound I formation. ▨ (+)-Catechin consumption.

Table 3 The effect of catalyst on the formation of phenol adducts.

Catalyst	Amount (mmol)	Tme (nim.)	Consumption[1] (mol %)	Formation (mol %) I	II	III	Selective[2] index
BF_3	0.45	30	99.3	56.3	3.5	6.8	56.7
		60	99.7	24.5	4.5	7.4	24.6
$TiCl_4$	0.45	30	85.8	1.1	0.6	19.1	1.3
		60	91.8	1.0	0.8	20.7	1.1
	0.045	30	91.8	2.1	n.d.	3.7	2.3
		60	92.8	2.4	n.d.	4.4	2.6
	0.023	30	83.4	1.8	n.d.	1.5	2.2
		60	84.9	1.7	n.d.	2.0	1.9
$SnCl_4$	0.45	30	95.0	5.7	2.0	1.7	6.0
		60	94.9	5.5	2.8	3.0	5.8
	0.045	30	75.3	n.d.	n.d.	n.d.	—
		60	82.1	1.6	n.d.	n.d.	1.9
$AlCl_3$	0.45	30	67.2	8.8	n.d.	5.7	13.1
		60	80.1	7.8	0.2	11.4	9.7
	0.23	30	58.3	3.0	n.d.	2.0	5.2
		60	74.1	1.1	n.d.	3.5	1.5
HF	0.45	30	43.0	n.d.	n.d.	n.d.	—
		60	45.9	n.d.	n.d.	n.d.	—
	2.30	30	36.0	1.6	n.d.	n.d.	4.3
		60	50.1	1.6	n.d.	n.d.	3.2
HCl	0.45	30	80.4	n.d.	n.d.	5.4	—
		60	83.1	n.d.	n.d.	7.2	—
H_2SO_4	0.45	30	93.0	7.0	0.6	11.2	7.6
		60	99.0	3.4	0.6	22.8	3.5
	0.23	30	74.1	10.5	0.6	12.6	14.1
		60	82.9	4.4	0.9	16.4	5.3
	0.11	30	73.4	5.2	0.2	3.5	7.1
		60	80.0	2.7	0.2	4.7	3.3
CH_3COOH	0.45	30	17.4	n.d.	n.d.	n.d.	—
		60	43.2	n.d.	n.d.	n.d.	—
	4.50	30	10.9	n.d.	n.d.	n.d.	—
		60	18.9	n.d.	n.d.	n.d.	—
CCl_3COOH	0.45	30	30.1	n.d.	n.d.	n.d.	—
		60	18.3	n.d.	n.d.	n.d.	—
	2.30	30	1.3	n.d.	n.d.	n.d.	—
		60	12.9	n.d.	n.d.	n.d.	—

* These reactions were conducted in benzene solution.

1) Consumption of (+)-catechin.
2) Selective index = [Formation of Compound I / Consumption of (+)-catechin] x 100

number of substituent groups on the aromatic ring of the solvents. The same tendency also was shown between anisole and p-methylanisole. In regard to aprotic solvents, for example in alkyl halides, Compound I was produced at the yield of more than 30mol% as well as in the case of aromatic solvents. On the other hand, little Compound I was produced in all protic solvents and in some aprotic solvents containing an oxygen atom such as ethylene glycol monomethylether and dioxane.

From these results, the following was considered about the effects of the solvents on the formation of Compound I. The BF_3 catalyst is stable in aromatic solvents because of its capability to form π complexes, very weak coordinates for delocalization of aromatic rings. Additionally, this ability to form the complex relates to the number of substituent groups on the solvents because of the steric hindrance between the BF_3 molecule and the solvents. On the contrary, BF_3 molecule was degraded in the protic solvents containing an oxygen atom as observed in water, because BF_3 coordinates strongly to a lone pair of electrons on the oxygen, and because this types of solvent has a solvation ability which can capture the nucleophilic reagent. In the aprotic solvents, however, nucleophilicity of phenol does not decrease because of the solvation. In general, the S_N1 reaction is promoted in the protic solvents since the carbocation is stabilized by the solvation. S_N2 reaction, on the other hand, is promoted in the aprotic solvents,[53] since hydrogen bonds do not form between aprotic solvents and nucleophilic reagents. Therefore, on considering the result of solvent effects, it is supported that the formation of compound I undergoes *via* S_N2 reaction.

Influences of the catalyst on the formation of Compound I

High catalytic activities of BF_3 on the formation of Compound I was understood from the above results. The catalytic capacities of other catalysts were examined to elucidate a

Table 4 The ion radius and interatomic distance of elements composing Lewis acid.

Ion radius (Å)		Interatomic distance (Å)	
B^{3+}	0.15	B-F	1.313
Ti^{4+}	0.75	Ti-Cl	2.170
Al^{3+}	0.53	Al-Cl	2.140
Sn^{4+}	0.69	Sn-Cl	2.281

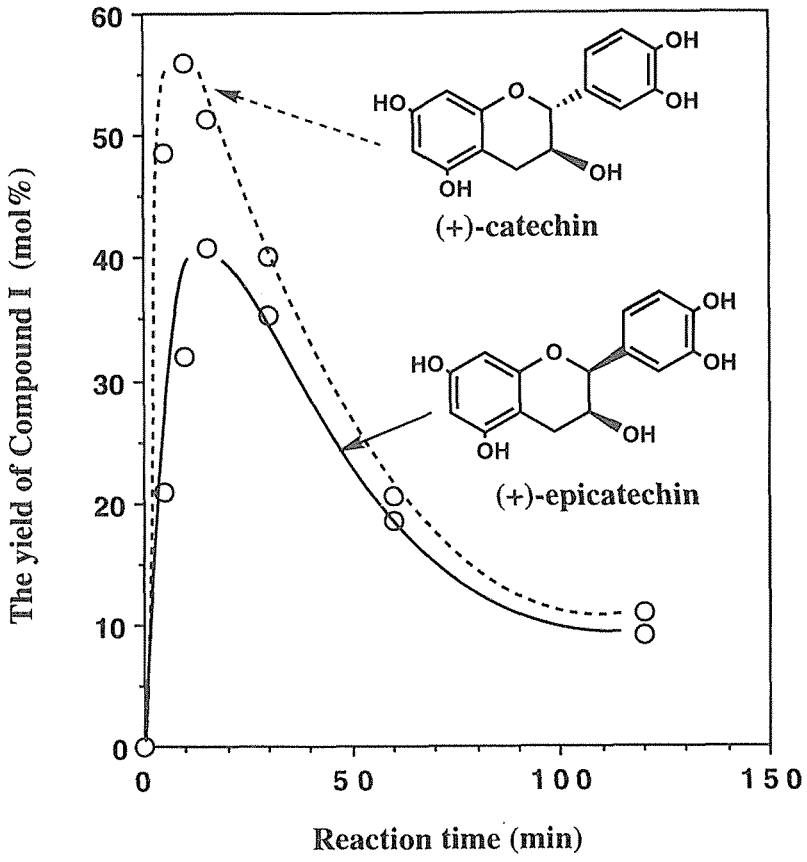


Fig. 17 The yield of Compound I from (+)-catechin or (+)-epicatechin.

Note: The reaction was conducted in a system in which 3 ml of reagent {benzene:phenol:phenol-BF_3 complex = 10:19:1 (v/v)} was added to 10 mg of (+)-catechin or (+)-epicatechin at 30 °C.

selective activity of BF_3 on the formation of Compound I. Table 3 shows the consumption of (+)-catechin and the formation of Compound I, II, and III. Additionally, the selective formation index (S.I.) of Compound I are calculated as follows; S.I.=(the amount of the formation of Compound I) x 100 / (the consumption of (+)-catechin). It was elucidated that Lewis acid except BF_3 or protic acid used were not as effective as BF_3 with respect to the formation of Compound I, and the high specific activity of BF_3 was indicated from the S.I. value.

The relationship between the formation of Compound I and the molecular size of Lewis acid used was investigated to explain the differences of catalytic activities on the formation of Compound I. The numerical values of interatomic distances of B-F, Ti-Cl, Sn-Cl, and Al-Cl are shown in Table 4.[54] The value of BF_3 is smaller than the other Lewis acids, which means that BF_3 molecule is easy to access to the oxgen atom on the pyran-ring of (+)-catechin. Therefore, it was indicated that the accessibility of the catalyst to the

reactive site of (+)-catechin was important interms of the formation of Compound I.

The formation of compound I from (+)-epicatechin

(+)-Epicatechin was synthesized from (+)-catechin in an ethanol solution at 180℃ for 1.5 hr. Because no peaks were detected except for (+)-epicatechin and (+)-catechin, it was obtained easily as white amorphous solid by preparative HPLC. Identification of (+)-epicatechin was done by a comparison of ^{1}H-NMR spectrum data which was reported by Foo and Porter.[52] Phenolation was done in a manner similar to that for (+)-catechin. As (+)-epicatechin is the C2 epimer of (+)-catechin, it was expected that diastereoisomer of Compound I could be obtained by this phenolation. Unexpectedly, Compound I was formed in the same manner as was (+)-catechin. This identification was made by checking with a standard sample of Compound I-Ac (acetate) in HPLC and ^{1}H-NMR data. However, a difference was observed in the amount of Compound I from these two isomers as shown in Fig.17. The maximum amount of Compound I from (+)-catechin reached about 55mol% in 10min; on the other hand, that amount from (+)-epicatechin reached about 40mol% in 15min. This indicated that the formation mechanisms of Compound I from these two isomers were different from each other.

Conformational analysis of (+)-catechin and (+)-epicatechin

Figure 18 shows the ^{1}H-NMR spectrum of the aliphatic region (2.2-5.2ppm) of (+)-catechin and (+)-epicatechin. In the case of (+)-catechin, axial and equatorial methylene protones of C4 are shown at 2.53 ppm as a double doublet (J=16Hz, 8.2Hz) and at 2.92ppm as a double doublet (J=16Hz, 5.3Hz), respectively. The C3 proton appears at 3.97-4.03ppm as a multiplet and the C2 proton does at 4.56ppm as a doublet (J=7.3Hz). On the other hand, in the spectrum of (+)-epicatechin, equatorial and axial methylene protones are shown at 2.74 ppm as a double doublet (J=16.8Hz, 3.3Hz) and at 2.87 ppm as a double doublet (J=16.7Hz, 4.5Hz), respectively. The C3 proton is shown at 4.10-4.26ppm as being broad and the C2 proton at 4.88ppm as a singlet. Judging from the relationships between coupling constants of the vicinal protons (2H and 3H) and their dihedral angle, it is considered that the dihedral angle of them is close to 180° (catechin) and 90° (epicatechin) as shown in the stereoviews in Fig. 18.

Formation mechanisms and stereochemistry of Compound I

In general, it is said that an intramolecular nucleophilic substitution (S_N) takes place with ease if a nucleophilic group, such as -OR[55], -OCOR[56], and -phenyl[57], is located to the anti-position to the leaving group. This substitution is known as the effect of neighboring participation.

As described above, a hydroxyl group at the C3 position is located anti-position against

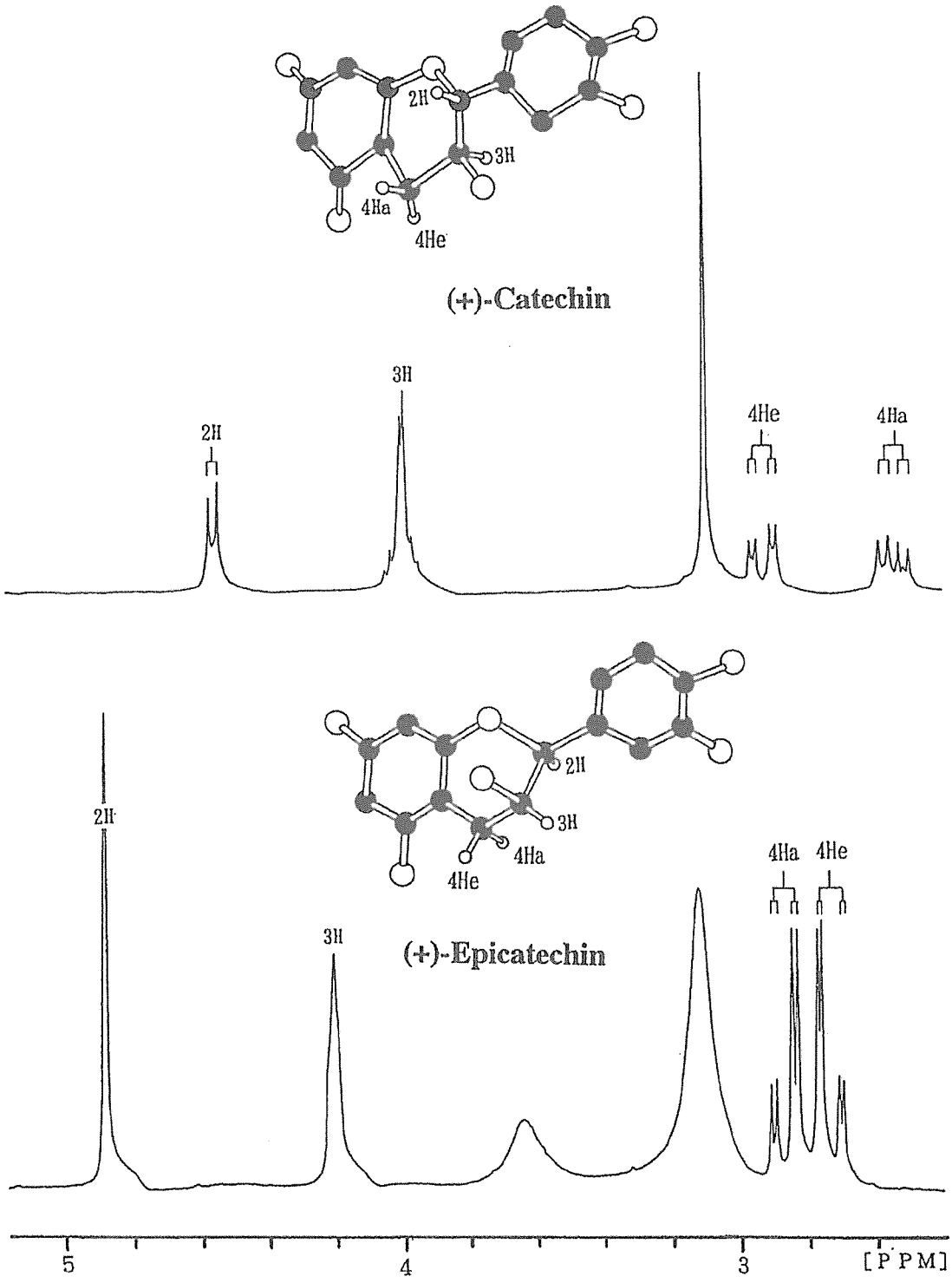


Fig. 18 Expanded aliphatic proton region of ^{1}H-NMR spectrum and stereoview of (+)-catechin and (+)-epicatechin.

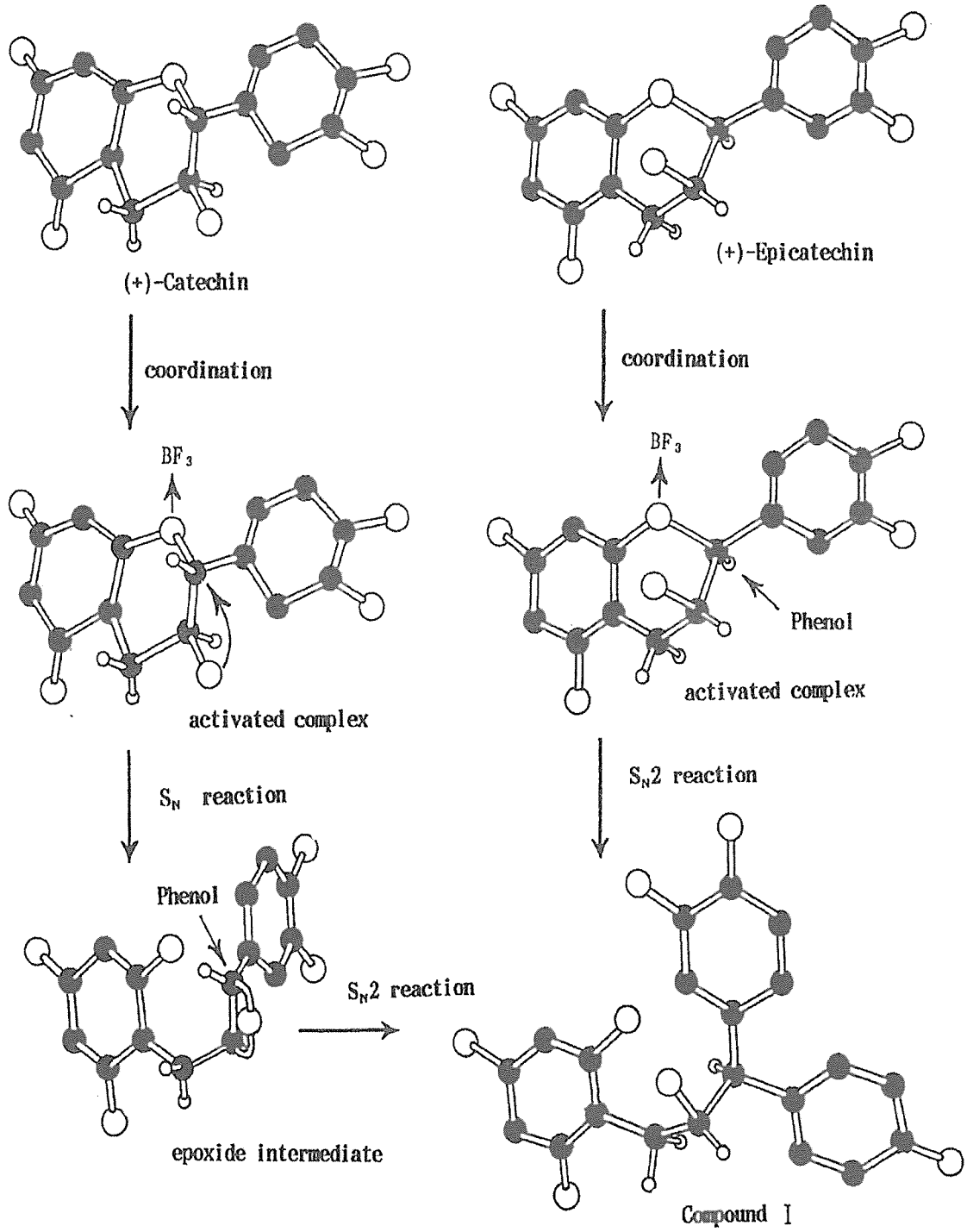


Fig. 19 The formation mechanisms of compound I from (+)-catechin or (+)-epicatechin.

the ether bond on the pyran ring in (+)-catechin and almost at the cis-position in (+)-epicatechin. Therefore, the phenolation mechanisms in the case of (+)-catechin is as follows: a lone pair of electrons of oxygen of the pyran ring coordinates to BF_3 first, and then the ether bond is cleaved at the same time that a lone pair of electrons of a hydroxyl group at the C3 position makes a nucleophilic attack on the C2 carbon to form epoxide intermediate. Successively, a phenol existing excessively makes a nucleophilic attack at the C2 carbon from the back side of the epoxide ring to form Compound I as shown in Fig. 19. Whereas, since (+)-epicatechin is not able to take the conformation to make an intramolecular nucleophilic substitution, that is a phenol attacks directly the C2 carbon from the back side against the ether bond to form Compound I. These ideas also are supported from the result in Fig. 17 as it is noted widely that the reaction which is subject to the neighboring group effect accelerates the reaction velocity. Thus, it should be considered that the formation of Compound I from (+)-catechin takes place mainly through two S_N2 reactions. Consequently, it is supported strongly that the absolute configuration of the C1 in Compound I is *R* configuration, because that of (+)-catechin is maintained as a result of two S_N2 reactions.

I-2-4 Summary

The effects of solvents on the formation of a ring-opened and phenolated product (Compound I), which was provided from (+)-catechin by BF_3 catalyst, was examined. Compound I was formed more in benzene than in any other solvents used, and its yield reached 56.3mol% based on (+)-catechin. Judging from the observations that this reaction took place in the aprotic solvents and not in the protic solvents, and Compound I also was formed from (+)-epicatechin in the same manner as (+)-catechin, the formation mechanisms of Compound I were expected to be as follows : 1) The C2 carbon of (+)-catechin was positively charged for the coordination to BF_3, and then the C2 carbon was attacked by unshared electron pair on oxygen in the C3 hydroxyl group for the neighboring group participation to form the epoxide intermediate. 2) Furthermore, the epoxide intermediate was attacked by a phenol molecule from the back side of an epoxide-ring to form Compound I. Since this reaction passed through a S_N2 reaction, the configuration of nucleophilic center in (+)-catechin was retained. Therefore, the absolute configuration at the C1 position of Compound I was estimated as an *R* configuration.

Chapter II
Analysis of the pyran-ring opening by the Methylation-Nucleus Exchange Reaction

II-1 Introduction

The modification of condensed tannins on pyran-ring opening has been undertaken by Pizzi and Roux as mentioned in PREFACE, however pyran-ring opened structure has not been identified though they have confirmed that the solution viscosity of the modified condensed tannins decreased in the preparaion of wood adhesives.

Thiolysis using a toluene-α-thiol is known as a popular analytical method for determing the composition of C4-C6 or C4-C8 isomer, the stereochemistry on pyran-ring, and the composition of aromatic rings of condensed tannins.[11,12,58-65] However, the results obtained by this method does not represent the composition of whole units, because the yields of degradation products are fairly small. On the other hand, Nucleus Exchange Reaction (NER) designed by Abe and Funaoka[46,66,67] is more reliable method to analyze the composition of A- and B-ring. In view of the principle, however, this method does not give an information about pyran-ring opening.

In this chapter, Methylation-NER (M-NER), the new method to analyze the frequency of pyran-ring opening for modified condensed tannins, is examined by using synthesized condensed tannins and also is evaluated by analyzing several natural condensed tannins.

II-2 Experimental

Procyanidin (PC) oligomer and profisetinidin (PF) dimer and oligomer were synthesized by the condensation of the corresponding flavan-3,4-diols and (+)-catechin under acidic conditions. (+)-Taxifolin and (+)-fustin were obtained from the heartwood of karamatsu (Larix leptolepis) and hazenoki (Rhus succedanea L), respectively. Apparatuses used for the analyses were as follows: ^{1}H-NMR, a Nihon-Denshi JNM-GSX 270 spectrometer; UV, a Nihon-Bunko UVIDEC-505; TLC was performed on Merck 60F254 sillica-gel precoated plates (solvent; toluene:ethyl formate:formic acid=5:1:4 v/v); GLC was performed by a Yanagimoto G-180 using a methyl silicone capillary column (Guadrex S2006, 0.25mm I.D. x 25m length x 0.25mm film thickness); HPLC was conducted by a Nihon-Bunko 800series using a Develosil packed column ODS-HG-5 (4.6mm x 250mm). The mobile phase was MeOH/0.01% trifluoroacetic acid=20/80; Gel permeation chromatography (GPC) was conducted with a Nihon-Bunko TRIROTOR using a Shodex packed column KF-802,804 (4.6mm x 250mm). The mobile phase was tetrahydrofuran. The calibration curve was made with (+)-catechin, PF dimer, and polystylene standards (molecular weights: 2000 and 9000).

Isolation of flavanonol

(+)-Taxifolin[68]

The tree sampled was a 30-year-old karamatsu growing in the Mie University Forest (Misugi, Mie prefecture) in August 1990. The heartwood sample was ground in a Wiley mill. The air-dried heartwood meal (1000g) was extracted with methanol (10l) for 24hr at ambient temperature. After evaporating the methanol, the residue was extracted with ether (500ml). The ether solution was evaporated to about 100ml under reduced pressure, and then the concentrated solution was stored in a refrigerator. Afterwards, a part (5g) of an ether soluble was separated into four fractions by column chromatography on Sephadex LH-20gel (2.5cm x 80cm). Crude crystal (1.0g) was isolated after several recrystallizations of the second fraction from hot water. It was identified by co-TLC and HPLC with authentic sample.

(+)-Fustin[69]

The tree sampled was an 80-year-old hazenoki growing in Wakayama Prefecture in Spring 1992. The heartwood sample was ground in a Wiley mill. The air-dried heartwood meal (500g) was extracted with methanol (7l) for 24hr at ambient temperature. After evaporating the methanol, the residue was extracted with ether (500ml). The ether solution was evaporated to about 100ml under reduced pressure, and then the concentrated solution was stored in a refrigerator. Afterwards, a part (5g) of an ether soluble was separated into three fractions by column chromatography on Sephadex LH-20gel (2.5cm x 80cm). Crude fustin crystal (1.3g) containing a small amount of fisetin was isolated. Fustin was identified by co-TLC and co-HPLC with authentic sample.

Syntheses of flavan-3,4-diols from flavanonols, (+)-taxifolin and (+)-fustin

Flavanonol (500mg) in EtOH (100ml) solution was stirred with $NaBH_4$ (500mg) for 4hr. The yellowish reaction mixture was poured into an excess of water, and the pH was adjusted to 3-4 with phosphoric acid. The solutions was extracted with EtOAc (100ml x 3), and the combined extract was dried over Na_2SO_4. Because flavan-3,4-diols are known to be very labile compounds under acidic conditions,[70] the extract was deacidified and chromatographed on a LH-20gel column by using water and ethanol as eluents, respectively. Leucocyanidin and leucofisetinidin were obtained in yields of 390mg and 425mg, respectively.

Preparations of synthesized-condensed tannins

Profisetinidin dimer

Leucofisetinidin (160mg) and (+)-catechin (160mg) were dissolved in 0.1N HCl (20ml) and the mixture was stirred at 25℃ for 24hr. Then, the reaction solution was deacidified with

a column (2.0cm x 45cm) of a Sephadex LH-20 in water and subsequently chromatographed with the same column in ethanol. The eluate was collected in 5ml aliquots. (−)-Fisetinidol-(4α→8)-(+)-catechin (32mg) and (−)-fisetinidol-(4β→8)-(+)-catechin (83mg) were isolated as light brown amorphous powders from fractions 25-38 and 55-83, respectively. The ^{1}H-NMR spectra of both compounds obtained and their acetylated derivatives were consistent with those previously reported.[71,72]

Procyanidin and profisetinidin oligomers

The condensation of the flavan-3,4-diol (150mg), leucocyanidin or leucofisetinidin, and (+)-catechin (30mg) was conducted in the same manner as the dimer. Reaction mixture was subjected to column chromatography on a Sephadex LH-20 pre-swollen in H_2O. The column was eluted with H_2O until HCl ran out of the eluent. Then the adsorbates were eluated by 70% acetone aqueous (aq.), and freeze-dried. The average degree of polymerization of PC and PF oligomers were determined by GPC as 7.2 and 4.8, respectively.

Preparation of natural condensed tannins

The commercially available extractives of wattle (w) and quebracho (Schinopsis lorentzii) (Q), and the 70% acetone aq. extractives of karamatsu (larix spp.) (K) and acacia (Acacia mearnsii) (A) were used as natural condensed tannins. These samples (4.0g) dissolved in 5ml of 70% acetone aq. were fractionated on a Sephadex LH-20 column (2.5 x 80cm) by eluting with 800ml of water, of ethanol (EE), of methanol Preparation scheme of these materials was shown in Fig 20.

Methylation-NER of condensed tannins

The sample (100mg), anhydrous potasium carbonate (1.0g) and dimethyl sulfate (700ml) in dry acetone (8ml) were refluxed for 2-3 hr. After removal of the inorganic salts, the filtrate was concentrated to a syrup, which was purified by silica gel chromatography with benzene : acetone (9 : 1) to furnish the methyl derivartives as a white amorphous powder.

The NER and purification of the products were conducted according to the method reported by Abe et al.[46] except that 20mg of sample and 300ml of reagent were used in the present experiment. Column temp. of GLC was maintained at 80℃ for 2.5min. and then increased to the final temp. 250℃ at a rate of 2℃/min.

Phenolations of synthesized-oligomers were conducted by the method described in the chapter I-1.

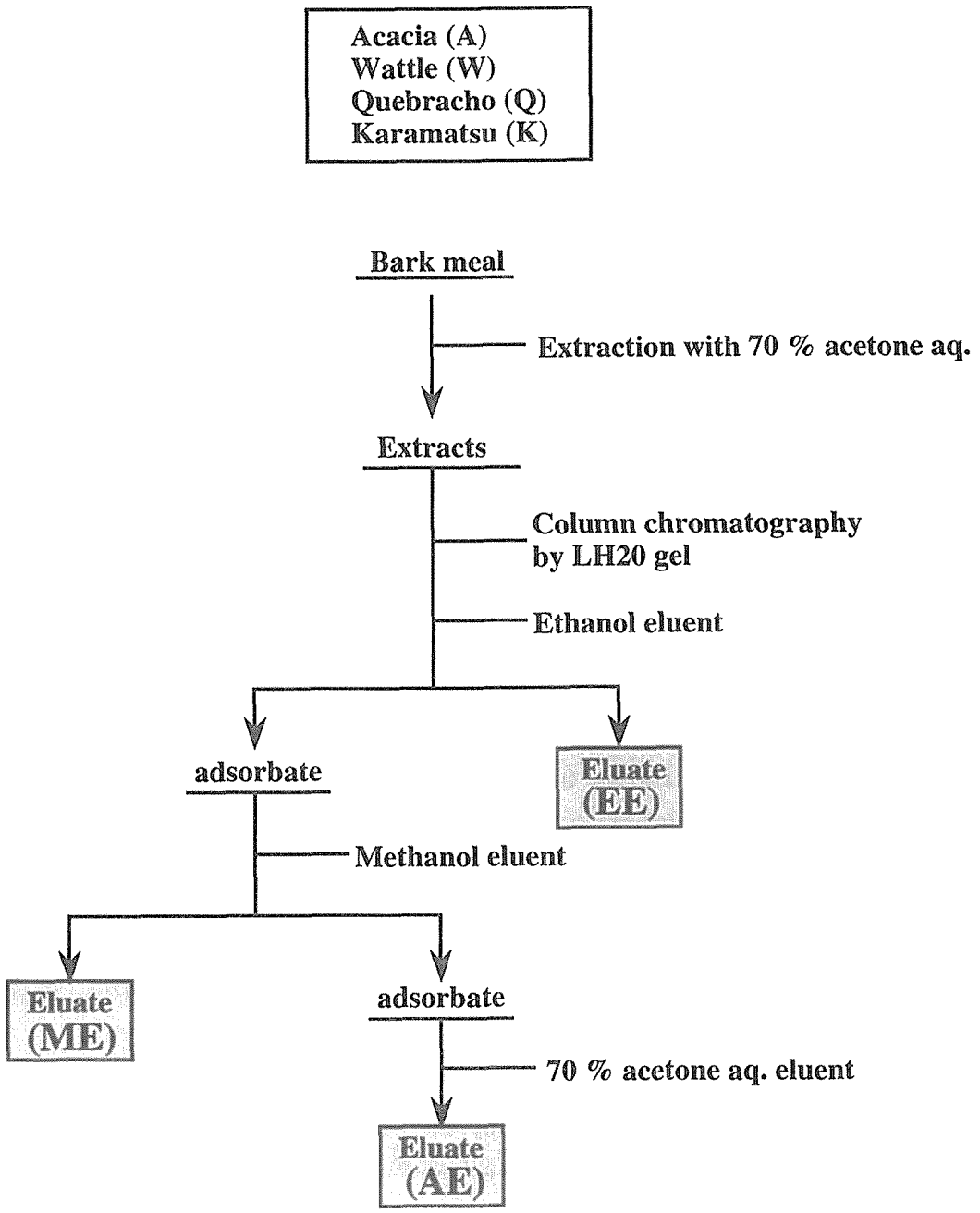


Fig. 20 Preparation of polyphenols from bark extracts.

II-3 Results and Discussion

Analysis of monomeric compounds by M-NER

NER is a method designed for the analysis of phenolic nuclei constituting lignin[67] and condensed tannins.[46] In the case of condensed tannins, phloroglucinol and resorcinol from the A-ring, and catechol and pyrogallol from the B-ring are liberated in large yields. Judging from this yield, the NER gives more information with regard to the phenolic nuclei constituting condensed tannins than does that by other degradation methods such as butanol-hydrochloric acid[73] or thiolysis.[12] The author thought that a nucleus liberated by NER of a methylated sample from a pyran-ring opened unit should be different from that liberated by the same method from a unit which is not opened. Thus, the M-NER method was theorized for the determination of the pyran-ring opening. That is to say, phloroglucinol trimethylether (PTE) and resorcinol dimethylether (RDE) are liberated from the pyran-ring opened units, and phloroglucinol dimethylether (PDE) and resorcinol monomethylether (RME) are liberated from the units holding the pyran-ring as shown in Fig. 21. In order to confirm the above hypothesis, the M-NER was applied to (+)-catechin holding the pyran-ring and to Compound I in which the pyran-ring was opened. Figure 22 shows GLC of the M-NER products obtained from methyl derivartives of (+)-catechin and Compound I. As presumed, PDE and PTE were produced from the above compounds, respectively. The yields of PDE and PTE are shown in Fig. 23. Both nuclei gradually increased with increase of the reaction time and reached about 90mol% per mole of their starting materials after 6 hour treatments of the NER. In our previous data,[74] only 25mol% of phloroglucinol was produced from (+)-catechin by the NER. This striking difference in the yields is presumably due to the stabilities of these liberated nuclei or intermediates liberating the nuclei in the reaction solution.

Investigation of the M-NER conditions by using profisetinidin dimer and oligomer

Two isomers of PF dimer were obtained by the condensation of (+)-leucofisetinidin and (+)-catechin as was reported by Young and others.[71] One of them, (+)-leucofisetinidol-(4 b6)-(+)-catechin, was used for the M-NER. Table 5 shows the yields of nuclei liberated from a mole of dimer at 60℃ or 80℃. RME and PDE were produced from the A-ring and veratrole (VER) from the B-ring because the dimer consists of a resorcinolic A-ring in the upper unit and a phloroglucinolic A-ring in the under unit, and catecholic B-rings in the both units. RME and PDE were produced in large yields, and the yield of 93.4mol% at maximum in PDE seems to be a fairly large quantity. On the other hand, VER was produced at about only 105mol% at maximum, in spite of producing 200mol% in theory. In addition, the formation of guaiacol (GUA) indicated that the demethylation of VER occurred in this reaction. Therefore, M-NER is a reliable method with regard to the analysis of nuclei present in the A-ring.

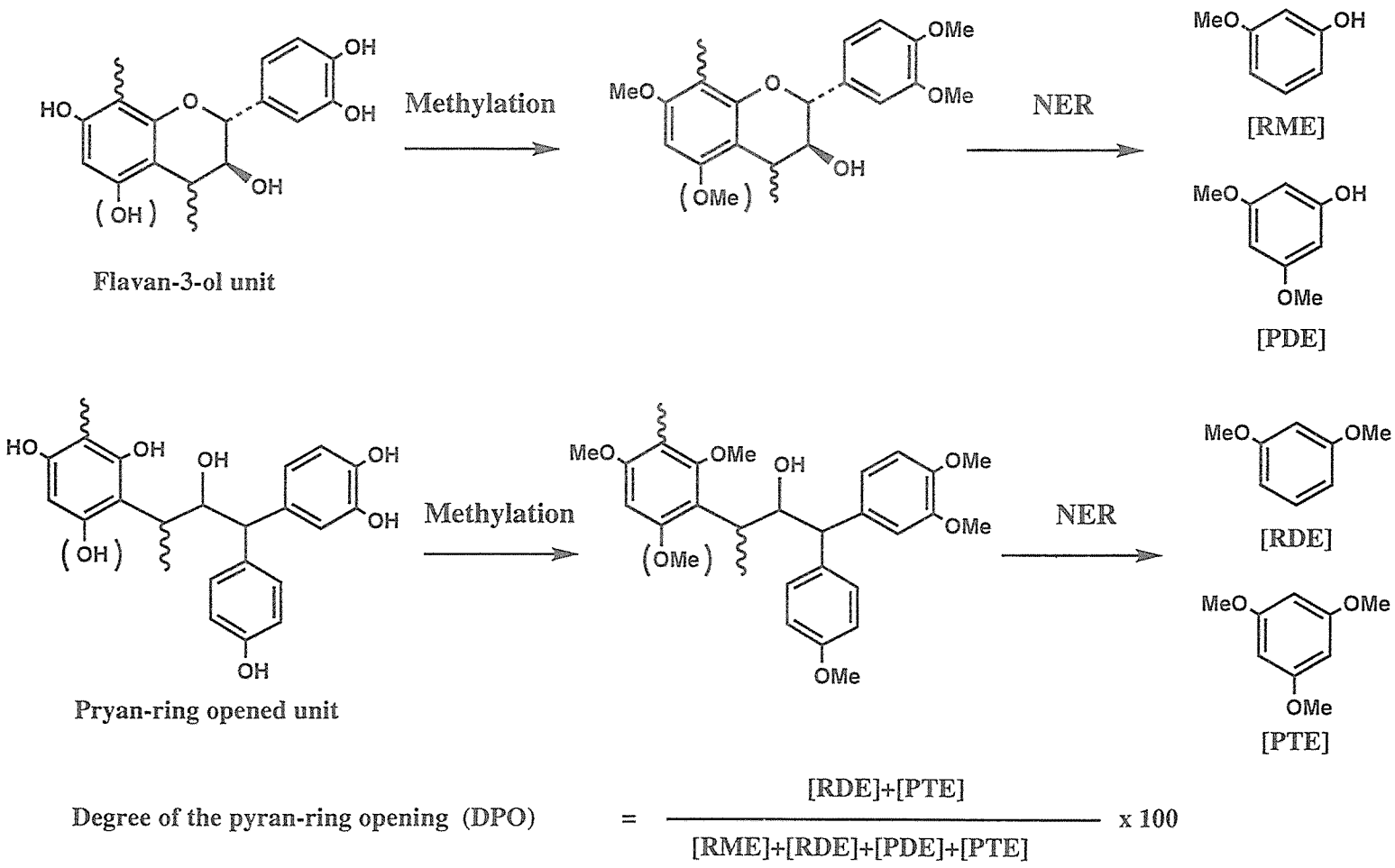


Fig. 21 The distinction between flavan-3-ol unit and pyran-ring opened unit by nuclear exchange reaction following methylation of condensed tannins.

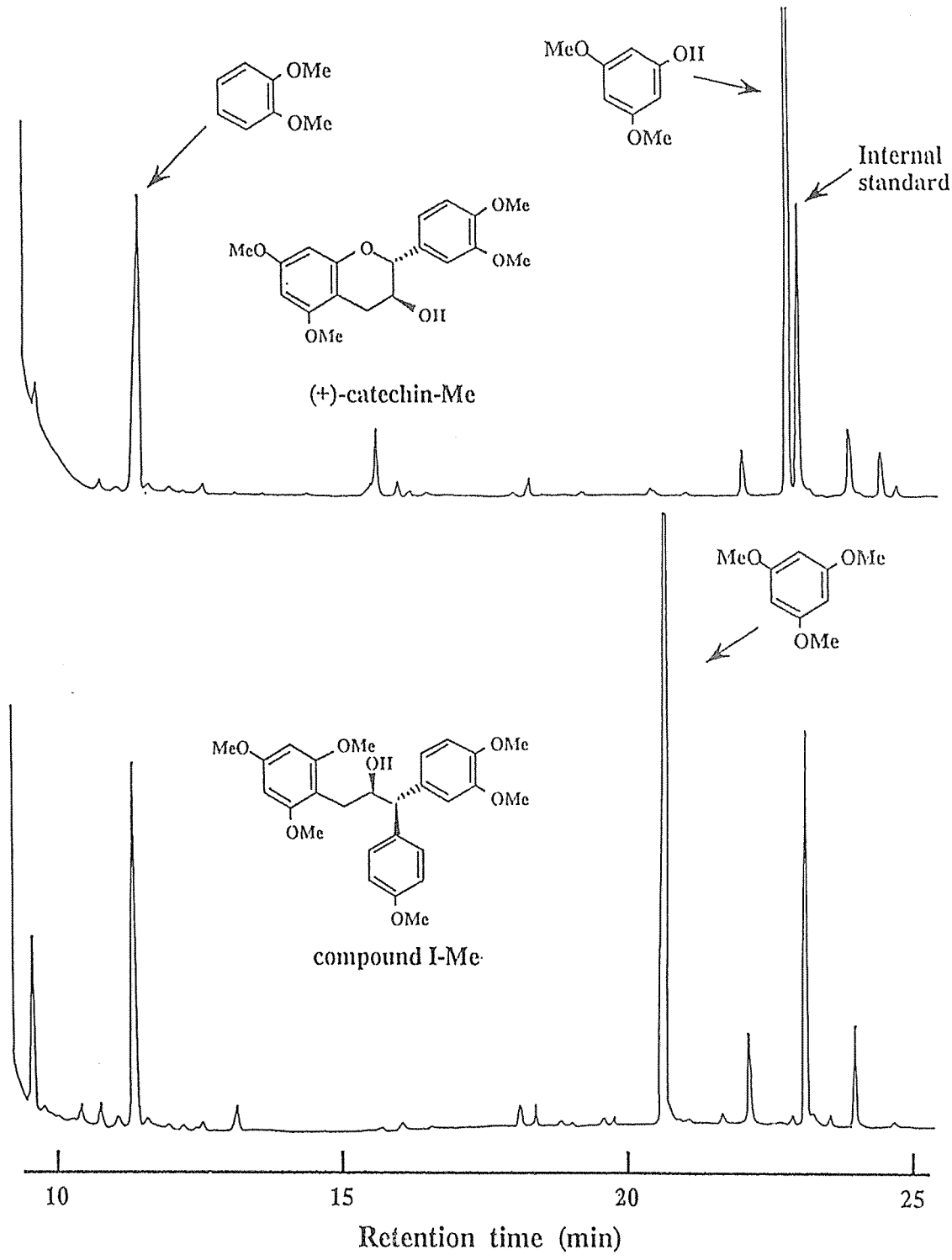


Fig. 22 GLCs of the products from NER of methyl derivatives of (+)-catechin and Compound I.

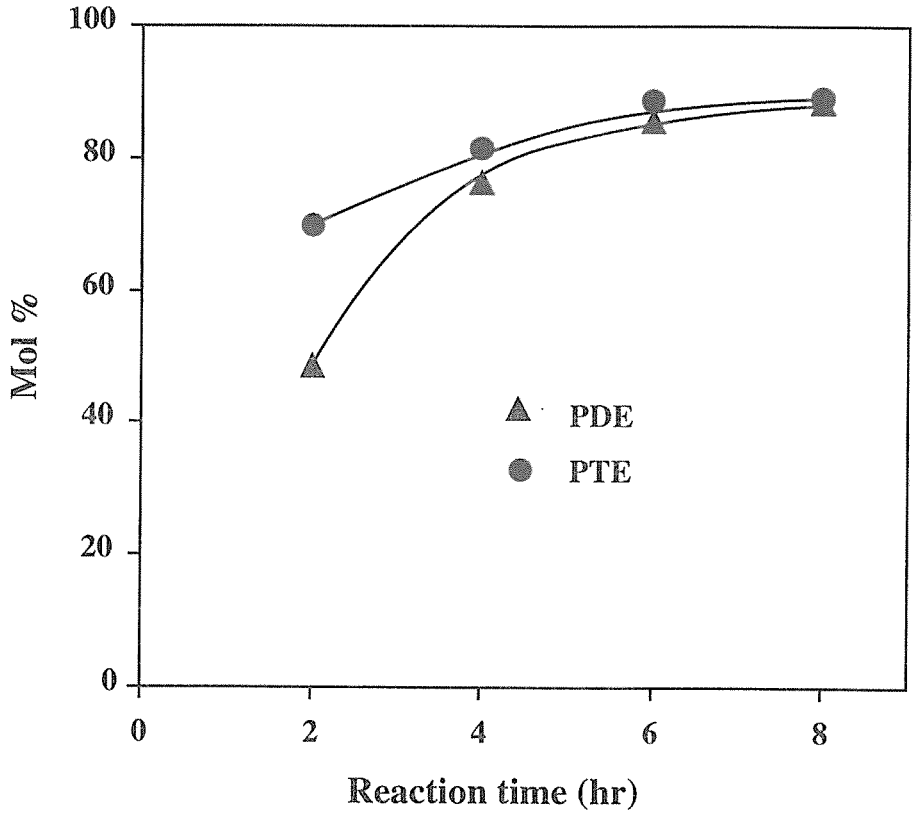


Fig. 23 The yields of nuclei liberated from methyl derivatives of (+)-catechin and compound I by NER at 60 °C.

Note; PDE: phloroglucinol dimethyl ether
PTE: phloroglucinol trimethyl ether

PF oligomer also was synthesized by the condensation of (+)-leucofisetinidin and (+)-catechin. This oligomer was estimated to be 4.8units in average degree of polymerization by the measurement of GPC, which imply 3.8extender units to a terminal unit in average. Table 5 shows the yields of nuclei liberated from methyl derivatives of the oligomer at 80℃, and is represented by mol% per a unit of fisetinidol methylether. The yields of RME and PDE showed 64.5mol% and 18.5mol% at maximum, respectively. Considering the degree of polymerization, these values were estimated as 81.5mol% (64.5 x 4.8/3.8) per a extender unit and 88.8mol% (18.5 x 4.8/1) per a terminal unit. Judging from the above results, the M-NER is an effective method to determine the degree of the pyran-ring opening not only in the extender units but also in the terminal unit of condensed tannins.

The analysis of natural condensed tannins by M-NER

Thiolysis has been used as one of the efficient methods for the structural analysis of

Table 5 **The yields of nuclei liberated from methyl derivatives of profisetinidin dimer and oligomer by NER at various conditions.**

[Mol %] *

Sample	Temp. (℃)	Time (hr)	A-ring				B-ring		
			RME	PDE	res.	phl.	VER	GUA	cate.
Dimer	60	4	76.6	82.9	0.6	n.d.	62.6	1.8	0.4
	60	6	77.5	86.7	0.7	n.d.	56.8	2.3	0.2
	80	2	86.7	93.4	0.6	0.5	96.9	6.9	0.5
	80	4	87.1	90.4	n.d.	1.0	104.9	11.3	0.4
	80	6	80.4	79.8	n.d.	1.2	98.3	15.3	1.0
Oligomer	80	2	64.5	18.5	1.2	0.8	43.9	3.3	0.1
	80	4	43.8	14.7	1.5	1.0	45.8	8.8	0.3

* Moles based on a mole of dimer methyl ether and a unit of fisetinidol methyl ether.

RME: resorcinol monomethyl ether VER: veratrole
PDE: phloroglucinol dimethyl ether GUA: guaiacol
res.: resorcinol phl.: phloroglucinol cate.: catechol

natural condensed tannins. This method gives information not only about the types of flavan-3-ol constituting condensed tannins,[75] but also of the stereochemistry of Sp3 carbons constructing the pyran-ring.[63] However, this information does not represent the whole analysis of materials used because the yields of the degradation products are very low.[76] On the other hand, that obtained by the NER and the M-NER were extremely high, so that these methods were applied to analyze almost all nuclei constituting natural condensed tannins. As mentioned above, the M-NER is an efficient tool for analysis of nuclei constituting the A-ring. Table 6 shows the yields of nuclei obtained from the A-ring by M-NER and those from the B-ring by the NER in several natural condensed tannins. As can be seen in wattle and acacia, A-rings are composed of phloroglucinolic and resorcinolic rings, and B-rings are composed of catecholic and pyrogallolic rings. Quebracho, on the other hand, is composed mainly of resorcinolic A-rings and catecholic B-rings. Furthermore, karamatsu is composed mainly of phloroglucinolic A-rings and catecholic B-rings as can be seen in general coniferous barks.[77] The nuclei of PTE and RDE, which have not been detected by M-NER in the synthesized dimer, were liberated from wattle, acacia, and quebracho, which indicates that these materials have the pyran-ring opened units. The degree of the pyran-ring opening (DPO) was calculated from {(PTE+RDE) / (PTE+PDE+RDE+RME)} x 100. In spite of the fact that wattle and acacia belong to the same species,

Table 6 The yields of nuclei liberated by M-NER from A-ring and NER from B-ring.

[mol %][a)]

Sample	A-ring[b)]						B-ring[c)]		
	RDE	RME	PTE	PDE	Total	DPO[d)]	catechol	pyrogallol	Total
W-ME[e)]	14.1	35.5	8.0	21.9	79.5	27.8	31.7	48.5	80.2
A-ME	4.1	42.3	8.2	23.5	78.1	15.7	28.7	49.5	78.2
Q-ME	47.8	23.1	2.6	4.3	77.8	64.8	77.3	3.0	80.3
K-AE	n.d.	n.d.	2.0	65.3	67.3	3.0	77.3	3.1	80.4

a) Moles based on a unit of flavan-3-ol and its methyl derivative.
b) M-NER was carried out at 80 °C for 2 hrs.
c) NER was carried out at 150 °C for 2 hrs.
d) DPO; Degree of the pyran-ring opening =[(RDE+PTE) / (RDE+RME+PTE+PDE)] x 100
RDE: resorcinol dimethyl ether PTE: phloroglucinol trimethyl ether
e) W, A, Q, and K are wattle, acacia, quebracho, and karamatsu, respectively.
ME and AE are methanol and 70 % acetone eluting fractions by
LH-20 gel column chromatography.

Table 7 The determination of DPO in the phenolation products of synthesized condensed tannin oligomers by M-NER.

Sample	Phenolation[a)] Time (min)	RDE	RME	PTE	PDE	Total	DPO(%)
PC-7.2[b)]	0	0	0	n.d.	82.3	82.3	0
	30	0	0	27.7	55.2	82.9	33.4
	120	0	0	41.6	44.1	85.7	48.5
	240	0	0	31.9	25.8	57.7	55.3
PF-4.8[c)]	0	n.d.	64.5	n.d.	18.5	83.0	0
	30	8.1	52.9	4.3	14.7	80.0	15.5
	120	8.5	53.5	4.4	13.8	80.2	16.1
	240	7.2	48.4	4.0	10.9	70.5	16.0

a) Phenolation was carried out at 40 °C.
b) The peak top in GPC of procyandin oligomer shows 7.2 in average of degree of polymerization.
c) The peak top in GPC of profisetinidin oligomer shows 4.8 in average of degree of polymerization.

the former has larger DPO than the latter. It will be assumed that this resullt is attributed to the differences of growing conditions of wattle in South Africa and acacia in Japan (Kumamoto prefecture).[78] In quebracho, it was indicated that about 65% of pyran-ring was opened. Although dimeric compound having the pyran-ring opened structure in an upper unit has been isolated from Uncaria gambir Roxb. belongs to Rubiaceae,[79] there has been no information with regard to such opened structure in wattle and quebracho.

The determination of the DPO in phenolated condensed tannin oligomers

Synthesized PF and PC oligomers were subjected to phenolation using BF_3 at 40℃. The M-NERs of the benzene insoluble parts in these phenolated oligomers were performed. The yields of nuclei liberated and the DPOs in phenolated oligomers are shown in Table 7. The DPO values of PC oligomers increased with the increase of phenolation time, and it was recognized that about 42% of the pyran-ring opened within 120min. On the other hand, the DPO of the PF oligomer showed about 16% constantly at all reaction times. As can be seen, the rate of the pyran-ring opening in the PC oligomer was faster than that in PF oligomer. Furthermore, the decreases of the total yields of the liberated nuclei with incresing phenolation times indicated that a part of phenolated oligomers passed into the benzene soluble part proceeding the phenolation. These behaviors of phenolation will be examined in detail in the next chapter.

As a result, it was demonstrated that the M-NER was an efficient method of analyzing the pyran-ring not only in the original condensed tannins but also in the phenolated tannins.

II-4 Summary

The nucleus exchange reaction of methyl derivatives (M-NER) of condensed tannins was examined to determine the degree of pyran-ring opening in their phenolated products. (+)-Catechin methyl ether having a pyran-ring and Compound I methyl ether with pyran-ring opening structure gave a phloroglucinol dimethyl ether and a phloroglucinol trimethyl ether in about 90% yields, respectively.

The M-NER was applied to the synthesized PF oligomer and several natural condensed tannins, and maximum yields of their nuclei were obtained at 80℃ for two hours. The opening structure of the pyran-ring was recognized in the natural condensed tannins by the M-NER method. Particulary, the 60% pyran-ring of the whole repeating units in quebracho tannins was estimated to open. Furthermore, 30-50% of pyran-ring in the PC oligomer opened during the phenolation at 40℃. It was explained that the rate of the pyran-ring opening of the PC type was faster than that of the PF type. The difference of the reaction rate was possibly due to the basicities of their A-rings as mentioned in other reaction such as thiolysis of condensed tannins.

Chapter III
The characteristics of the phenolation products derived from condensed tannins

III-1 Phenolation products and their reactivities with formaldehyde

III-1-1 Introduction

Several attempts to utilize natural condensed tannins as cold-setting wood laminating adhesives have been made by some researchers.[28,39,42,43] In the reaction of loblolly pine extracts and resorcinol under acidic condition, as mentioned by Hemingway, the reaction mixture containing predominantly oligomeric procyanidin-4-resorcinol adducts was used as a resorcinol replacement in a conventional phenol-resorcinol-formaldehyde laminating adhesives. Furthermore, it was indicated that the room temperature setting adhesives for wood-laminating purpose could be produced successfully using the adducts. In this process, however, condensed tannins were not used independently but were merely used as replacements of resorcinol which is one of the expensive phenols.

On the other hand, the author has expected that pyran-ring opening of condensed tannins leads to a flexible molecules to produce a modified condensed tannins having functions. A model experiment for modification of condensed tannins to open their pyran-ring by using BF_3 in excess cheap phenol was examined in chapter I. As the result, some phenolated products containing pyran-ring opened were produced in a large yield from (+)-catechin. The formation mechanisms were discussed on the basis of the stereochemistry of the products. Furthermore, the analytical method of opening the pyran-ring was developed.

In this chapter, the behavior with respect to the opening of the pyran-ring and the cleaving of the interflavanoid bonds of natural condensed tannins under phenolation in the presence of BF_3 are described. Additionally, the reactivity of phenolated condensed tannins with formaldehyde is also disccussed.

III-1-2 Experimental

Materials

Commercially available (+)-catechin, boron trifluoride-phenol complex, formaldehyde, and other chemical reagents were used. Compound I, having the pyran-ring opened structure, was synthesized from (+)-catechin according to the method described in chapter I-1. The seventy percentage acetone aqueous (aq.) extracts (AE) of acacia (Acacia

mearnsii) and karamatsu (Larix leptolepis) barks known as sources of condensed tannins were used in this study. Bark meal (500g) of karamastu was extracted successively with petroleum ether (3L) and diethyl ether (3L) for 48 hours at ambient temperature. Then, the residue was extracted with 70% acetone aq. (5L) with stirring for 48 hours at ambient temperature. After the acetone in the solution had evapolated, the AE was freeze-dried. The yield of AE obtained was 35% in acacia and 16% in karamatsu based on the bark meal.

Phenolation of AE

Dried AE powder (20mg) was added to a glass ampule with phenolation reagent (1ml), phenol : benzene : BF_3-phenol complex=19 : 10 : 1 v/v, and was heated in a water bath at 40℃. After the phenolation having been completed, the reaction mixture was poured into water (200ml) and then extracted with ethyl acetate (15ml x 3). A part of the ethyl acetate solution was used for the determination of liberated phenyl nuclei by HPLC, which was conducted on a JASCO 800 series system (column: Develosil ODS-HG-5 4.6mmf x 250mm, eluent: MeOH / 0.01% trifluoroacetic acid (TFA) aq.=22 / 78 →33 / 67 linear gradient for 40min, detector: UV 280nm). The remainder of the solution was added dropwise into benzene (50ml) to obtain the phenolated products as brownish precipitates. The precipitates are expressed as benzene insoluble (BI) fraction in this thesis.

Analyses of BI fraction

Nucleus exchange reaction of methyl derivatives (M-NER) of BI fraction were conducted as described in chapter II. Gel permeation chromatography (GPC) was recorded with a Jasco Trirotar system with Shodex GPC columns KF-802 and KF-804 (4.6mmf x 250mm) using tetrahydrofuran as an eluent. The chromatogram was calibrated against standard polystyrenes (Molecular weihts: 2000 and 9000), (+)-catechin, and synthesized PF dimer. Average of molecular weights (Mn, Mw) were calculated by an integrator, Jasco 807-IT, from the molecular weight distributions obtained by the GPC method.

Reaction with formaldehyde

About 0.07mmol of (+)-catechin or Compound I was dissolved in 5ml of ethanol containing 0.21mmol of formaldehyde. Five ml of 0.1N hydrochloric acid was added to the solution. The reaction was carried out at 25℃ in a vial with stirring. The reaction mixture was poured into excess water, and extracted with ethyl acetate (15 ml x 3). Nalingenin, the internal standard was added to the ethyl acetate solution, and then consumption of the starting material was determined by HPLC analysis.

About 30mg of AE was dissolved in 1.5ml of ethanol containing about 0.3mmol of formaldehyde. One and half ml of 0.1N hydrochloric acid was added to the solution. The reaction was carried out at 25℃ in a vial with stirring. The reaction mixture was subjected

to column chromatography on a Sephadex LH-20 gel using ethanol as an eluent to remove hydrochloric acid, the remaining formaldehyde, and low molecular weight materials. Adsorbates on the column were eluated with 70% acetone aq. to give the reaction products. Average of molecular weights of the products were calculated by the same method as described above.

III-1-3 Results and Discussion

The phenolation behavior of the AE fractions of acacia and karamatsu

From our results of NER analyses[80] condensed tannins in acacia and karamatsu mainly consist of resorcinolic and phloroglucinolic A-rings, respectively. The phenolations of these materials were made at 40℃ in the presence of BF_3, and the reaction mixture was added dropwise into benzene to eliminate the large excesses of phenol and phenolic nuclei liberated in the process of the phenolations. The yields of the BI fractions in their phenolated products are shown in Fig. 24, and Table 8 shows the amounts of nuclei liberated during the phenolations. The drop in yield of the BI fraction in karamatsu was significantly larger than that of acacia. Furthermore, an increase in the amount of liberated nuclei in karamatsu was seen as the phenolation proceeded. These results indicate that the degradation took place during the phenolation in karamatsu particularly,

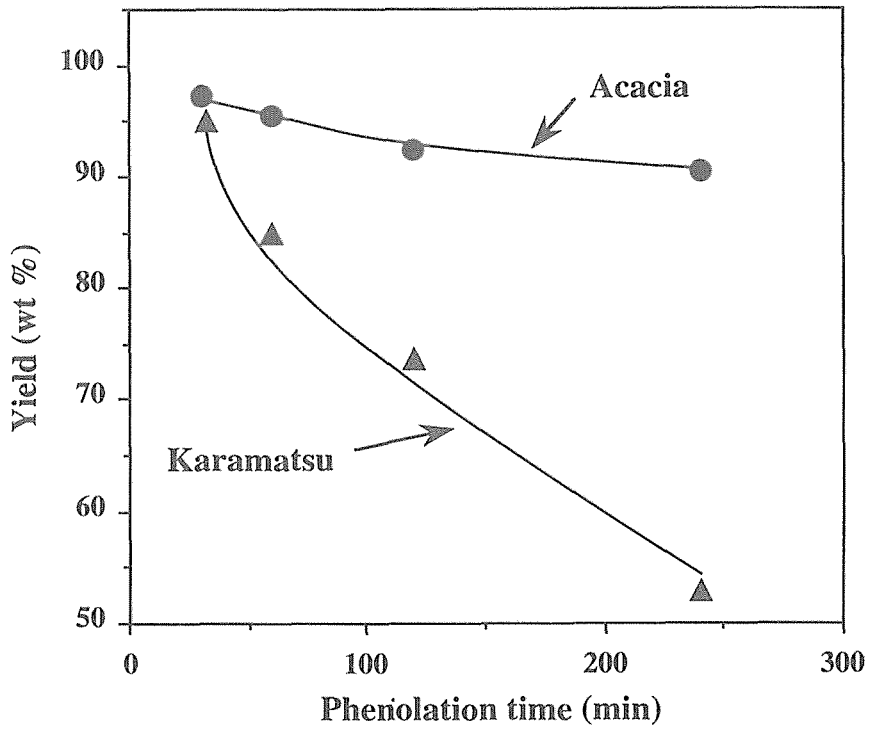


Fig. 24 The yield of benzene insoluble fraction in phenolated products at 40 °C.

Table 8 The yields of nuclei liberated in the process of the phenolation.

[mol %][a)]

Sample	Phenolation time (min)	A-ring	
		resorcinol	phloroglucinol
Karamatsu	30	n.d. [b)]	3.9
	60	n.d.	5.3
	120	n.d.	9.3
	240	n.d.	12.5
Acacia	30	0.9	n.d.
	60	1.8	1.2
	120	2.9	2.0
	240	5.3	3.5

a) Mole percent of each nucleus per a flavan-3-ol unit.
b) Not determined.

and that products shifted into benzene soluble (BS) fractions. GPCs of BI and BS fractions obtained from the products in the reactions after 30 and 240min are shown in Fig. 25. The molecular weights of phenolated products in BI fractions decreased gradually with increases of phenolation time. In karamatsu, particularly, rapid lowering of molecular weights of condensed tannins was observed within 30min of reaction time, and then the decreases in molecular weights occurred at slower rates than those during the initial 30min. Degradation products with molecular weight of less than 300 were fractionated in BS fractions. Taking into account the fact that karamatsu AE consists of only phloroglucinolic A-rings, but, acacia has mainly resorcinolic nuclei, it showed that the interflavanoid bonds of condensed tannins consisting of phloroglucinolic A-rings are more labile than those of resorcinolic A-rings by this phenolation.

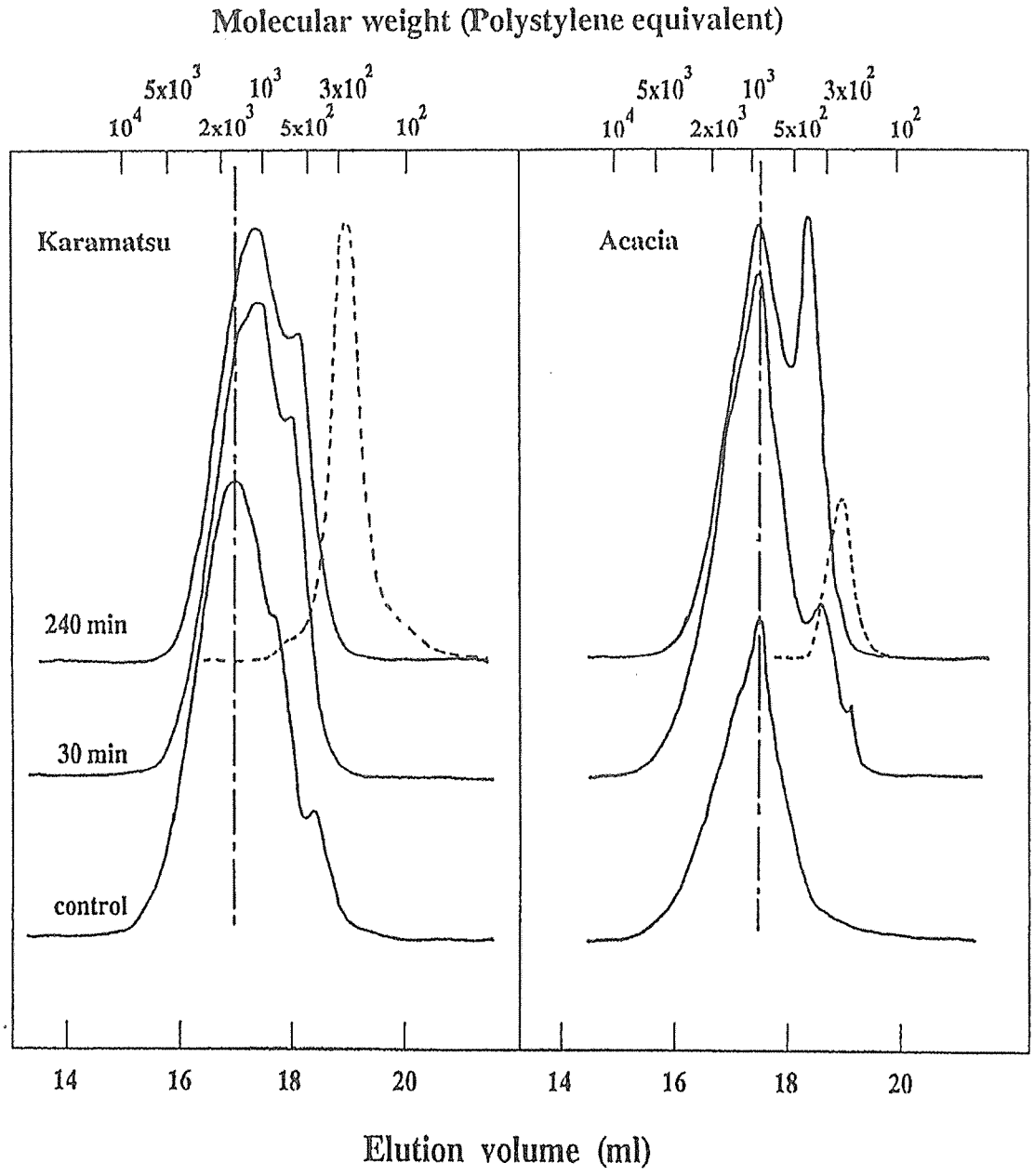


Fig. 25 GPC elution curves of phenolated condensed tannins

Legend: —— benzene insoluble fraction ····· benzene soluble fraction

Note: Times in figure represent phenolation time.

Table 9 Degree of the pyran-ring opening in phenolation products of condensed tannins in acetone water extracts.

[mol %][a)]

Sample	Phenolation time (min)	A-ring[b)]					DPO(%)[c)]	
		PTE	PDE	RDE	RME	Total	PU	RU[d)]
Karamatsu	0	1.0	44.2			45.2	2.2	
	30	8.4	26.8			35.2	23.9	
	120	7.6	18.8			26.4	28.8	
	240	7.2	12.0			19.2	37.5	
Acacia	0	0.9	11.6	3.5	29.5	45.5	7.2	10.6
	30	4.3	11.4	2.5	24.6	42.8	27.4	9.2
	120	3.3	8.3	1.7	16.3	28.3	28.4	9.4
	240	2.7	7.0	1.6	15.0	24.7	27.8	9.6

a) Mole percentage of each nucleus per a unit of flavan-3-ol methyl derivatives.
b) PTE: phloroglucinol trimethyl ether PDE: phloroglucinol dimethyl ether
RDE: resorcinol dimethyl ether RME: resorcinol monomethyl ether
c) DPO: Degree of the pyran-ring opening .
d) PU: phloroglucinolic unit = PTE / (PTE + PDE)
RU: resorcinolic unit = RDE / (RDE + RME)

Opening of the pyran-ring in the phenolation process

The M-NER of condensed tannins is used as an efficient method for the analysis of their pyran-ring opening as described in chapter II. This analytical method was applied to the BI fractions of phenolated condensed tannins. Table 9 shows the yields of nuclei liberated by the M-NER and the degrees of the pyran-ring opening (DPO) in karamatsu and acacia. The DPO values of phloroglucinolic nuclei increased extensively in both materials, but these of resorcinolic nuclei changed little during the phenolation. Judging from the action of BF_3 in the phenolation, these results indicate that the flavan-3-ol unit consisting of phloroglucinolic A-rings has a greater ability of coordinating a lone pair of electrons on the oxygen atom of a pyran-ring for BF_3 than does resorcinolic A-rings. However, total yields of each nucleus decreased with increases of phenolation time in spite of an increase of the DPO. Therefore, a short period of phenolation is required for obtaining a reactive phenolation products keeping many nuclei constituting the A-ring and that having the pyran-ring opened structure.

The reaction of (+)-catechin or compound I with formaldehyde

(+)-Catechin and Compound I were used to examine the reactivity with formaldehyde under acidic conditions, the former has a flavan-3-ol structure and the latter has a pyran-ring opened structure. Compound I was synthesized from (+)-catechin in a large yield by the phenolation in the presence of BF_3. The ln(a-x)/a derived from the amount of starting materials used and their consumption are plotted against the reaction times in Fig.

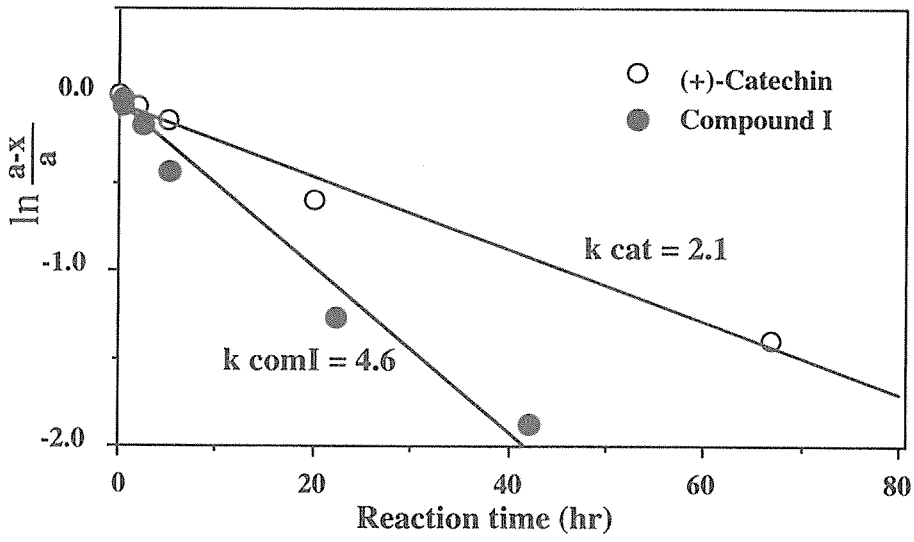


Fig. 26 Velocity of the consumption of (+)-catechin and Compound I on the reaction with formaldehyde in acidic condition at 25 °C.

Note: a: amount of starting materials used, x: consumption
k: rate constant (hours^{-1})

26. These plots are linear, showing pseudo first-order reactions with respect to their concentrations. Furthermore, the rate constant (k) of Compound I is about 2.2 times that of (+)-catechin. These results show that the opening of the pyran-ring influences the increase of the rate of reaction with formaldehyde. In this reaction, the product of bis-8,8′-catechinylmethane from (+)-catechin was confirmed by the comparison with the 1H-NMR data of authentic sample which was produced from the reaction of (+)-catechin and formaldehyde under basic conditions by Kiatgrajai's method.[81] Therefore, the consumption of (+)-catechin or Compound I would mean to form oligomeric or polymeric condensation products with methylene bridges.

The reaction of phenolated karamatsu AE with formaldehyde

In general, resorcinol formaldehyde resins are prepared under acidic or neutral conditions at ambient temperature to form novolak type adhesives.[82] The reactions of

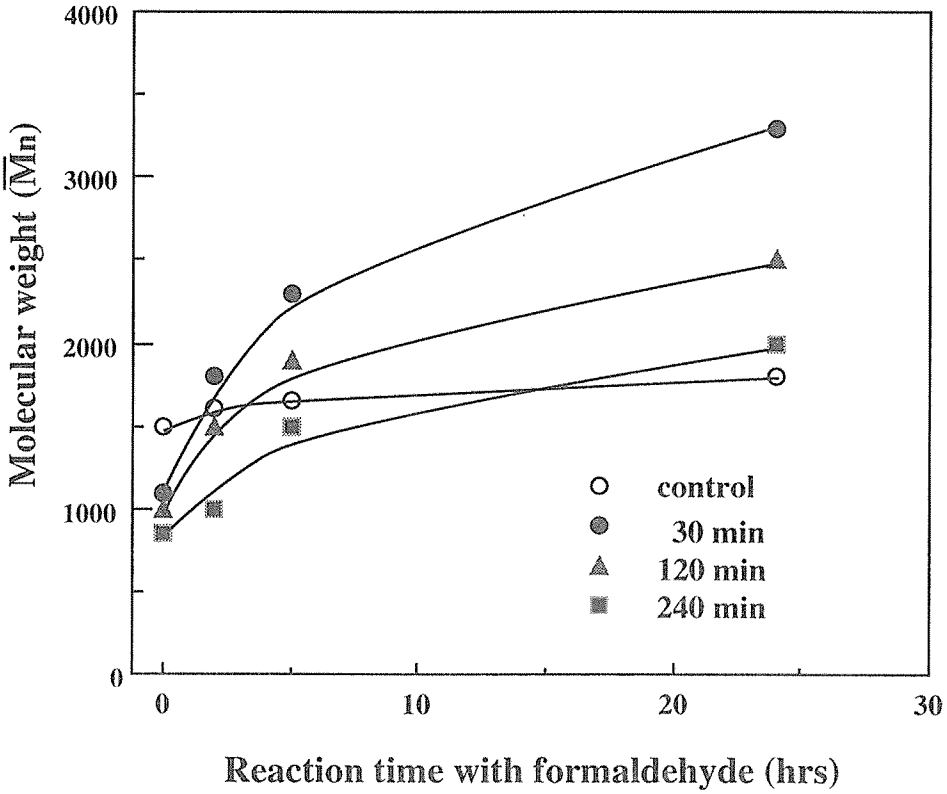


Fig. 27 Changes of molecular weight of the products in the reaction of phenolated karamatsu bark extracts with formaldehyde.

Note: Times in figure represent the treatment time of phenolation. Control is 70 % acetone aq. extracts of karamatsu.

polymeric polyphenols with aldehydes in several pH ranges have been examined,[83-86] and it is generally accepted that the reaction rate is slowest in the pH range 4.0-4.5.[87] In this study, the condensation of phenolated karamatsu AE (PKA) with formaldehyde (F) was examined under conditions of pH 3-4 at 20℃, and the molecular weights of the condensation products of PKA with F (PKA-F) against condensation times are shown in Fig.27. As can be seen, the molecular weight of the control, the reaction product of karamatsu AE with formaldehyde (KA-F), increased slowly. On the other hand, that in the PKA-F increased rapidly and reached three times the molecular weight compared with prior to the condensation after 24hr. The molecular weight of PKA-F decreased with increses of the treatment time in phenolation because the amount of phloroglucinolic nuclei remaining in the A-ring decreased during the phenolation, judging from the yields of liberated nuclei shown in Table 8. Takano88) found that a dimeric flavanol has a better bonding strength and a lower viscosity than does a polymeric flavanol in karamatsu and sugi tannins, and he concluded that the reduction of high molecular weight polyphenols was useful in the production of cold-setting adhesives.

Therefore, phenolation of condensed tannins in the presence of BF_3 seemes to be a suitable technique for decreasing the molecular weights of condensed tannins as mentioned above, and additionally, it also increases the rate of reactions with formaldehyde by their pyran-ring openings.

III-1-4 Summary

The phenolations of bark extracts with 70% acetone aq. from karamatsu and acacia were examined in the presence of excess phenol and boron trifluoride (BF_3) at 40℃. It was found that both the pyran-ring opening and the interflavanoid bond cleavage of karamatsu condensed tannins consisting of phloroglucinolic A-rings proceed more rapidly than those of acacia mainly consisting of resorcinolic A-rings.

In the condensation with formaldehyde under acidic conditions at 25℃, the consumption rate of Compound I which is a phenol adduct with the pyran-ring opened structure and, increased 2.2 times compared with that of (+)-catechin. Furthermore, in the same reaction of the phenol adduct with the pyran-ring opened structure produced from karamatsu extracts, the cross-linking proceeded to increase the molecular weight more than three times compared with that of phenol adduct within four hours reaction time.

Therefore, it was indicated that the modification, such as the pyran-ring opening and the interflavanoid bond cleaving, could improve condensed tannins into the reactive molecule to increase the rate of reaction with formaldehyde.

III-2 Inhibitory effects of the phenolated condensed tannins on glucosyltransferase activity

III-2-1 Introduction

Bark is the defensive tissue against attacks of wood-rotting fungi or insects, and bark polyphenols are believed to play an important role in the defensive actions.[89] This idea is thought to be intimately related to the protein precipitation ability and enzyme inhibition of polyphenols. Hydrolyzable tannins especially are known to inhibitor of the enzyme including trypsin,[90] pectin estelase,[91] and β-glucosidase[92] and also condensed tannins inhibit proteases[93] such as leucine aminopeptidase and chymotrypsin.

The author found that condensed tannins originated from woody bark inhibited the synthesis of insoluble glucan by cell-bound glucosyltransferase (GTase). Because glucan synthesis is regarded as intimate relating with a dental caries, the function of condensed tannins to prevent the dental caries was expected. Hydrolyzable tannins from crude drugs also inhibited GTase[94] and the inhibition was enhanced as increasing the ratio of galloyl / glucose in the tannins.[95] The chemical structure of tannins, thus, influence the degree of GTase inhibition, and in the case of condensed tannins, it is considered that ortho-hydroxyl group of B-ring participates in that inhibition. Therefore, it is expected that phenolated condensed tannins with the pyran-ring opened structure, which have more flexible structure than original ones, would inhibit highly the GTase activity.

In this chapter, inhibitory effects on GTase of phenolated (+)-catechin or natural condensed tannins modified by using BF_3 and excess phenol is described.

III-2-2 Experimental

Materials

Seventy % acetone aq. extracts of acacia and karamatsu barks, and commercially available quebracho extracts were fractionated by LH-20 gel column chromatography as described in chapter II, and then the eluate fractions (EE, ME, and AE) were used as natural polyphenols. PC and PF dimers were synthesized according to the method in chapter II. Additionally, the phenolated products (BI fraction) were preparated from the above natural materials (ME fraction) by the same methods in the section III-1, and Compound I was synthesized according to a method in chapter I as a model compound having the opened pyran-ring.

Analytical methods

Flavanol contents of natural polyphenols were determined according to the vanillin-HCl method described in the following way. One ml of methanol solution dissolving 0.5mg−1.0mg of polyphenols was added to 6ml of 4% vanillin methanol solution and 3ml of conc. HCl in

a brown test tube. After 15min of stirring, the absorbance at 500nm of the reaction mixture was measured with a Jasco UVIDEC-505 spectrophotometer. The calibraion curve was made using (+)-catechin as a standard sample.

The measurement of GPC and the calculation methods of Mn and Mw were conducted in the same manner as described in III-1-2.

Preparation of GTase

Streptococus sobrinus 6715 was grown for 16 hr at 37℃ in 5 l of Todd Hewitt (TH) broth. After the liquid medium having been centrifuged at 5000rpm for 15min, the mycelium was collected and then extracted with 75ml of 8M urea at 20℃ for 1hr with stirring. The crude enzyme solution containing urea was dialyzed against 10mM potassium phosphate buffer (pH 6) until the urea was removed entirely. After then, 1 ml of the crude enzyme solution was pipetted into a microtube, and stored in a freezer at −80℃.

Assay for GTase inhibitory activity

Insoluble glucan synthesized by GTase was measured turbidimetrically with a spectrophotometer by determining the increase in A550. GTase was incubated in 3ml of 0.1M phosphate buffer (pH 6.0) containing 1% sucrose, 0.1% sodium azide, 0.5% dextran T10, and polyphenols at 37℃ for 3hr. The volume of GTase solution used in the assay was determined by that giving absorbance 1.0 at 550nm. Inhibition rate is expressed by the following equation: Inhibition rate (%)=100 x (Ac-Ap)/Ac here, Ac and Ap represent absorbance obtained in control and in polyphenol dose, respectively. IC_{50} means the polyphenol concentration (μg/ml) giving 50% inhibition of GTase.

III-2-3 Results and Discussion

Inhibitory activity of the bark polyphenols

Green tea and oolong tea are known as the preventive beverages of dental caries, and the inhibition of GTase on polyphenols contained in these beverages were examined recently.[96-99] In those papers, the polyphenols from a oolong tea inhibited GTase ten times more than that from a green tea. Since oolong tea produced by a semi-fermentation from green tea is subjected to the structural changes by enzymes in that process, the oolong tea polyphenols are thought to be the oligomers converted from chatechins including epigallocatechin gallate in green tea. Then, GTase was presumably inhibited by bark polyphenols consisting of flavan-3-ol oligomer.

The relationship between the concentration of polyphenols from different sources and their inhibition rate on GTase is shown in Fig. 28. Both extracts from acacia and karamatsu exhibited higher inhibition rates than oolong tea extracts as expected. As can be seen in IC_{50}, inhibitory effect of karamatsu extracts particularly were about ten times that of

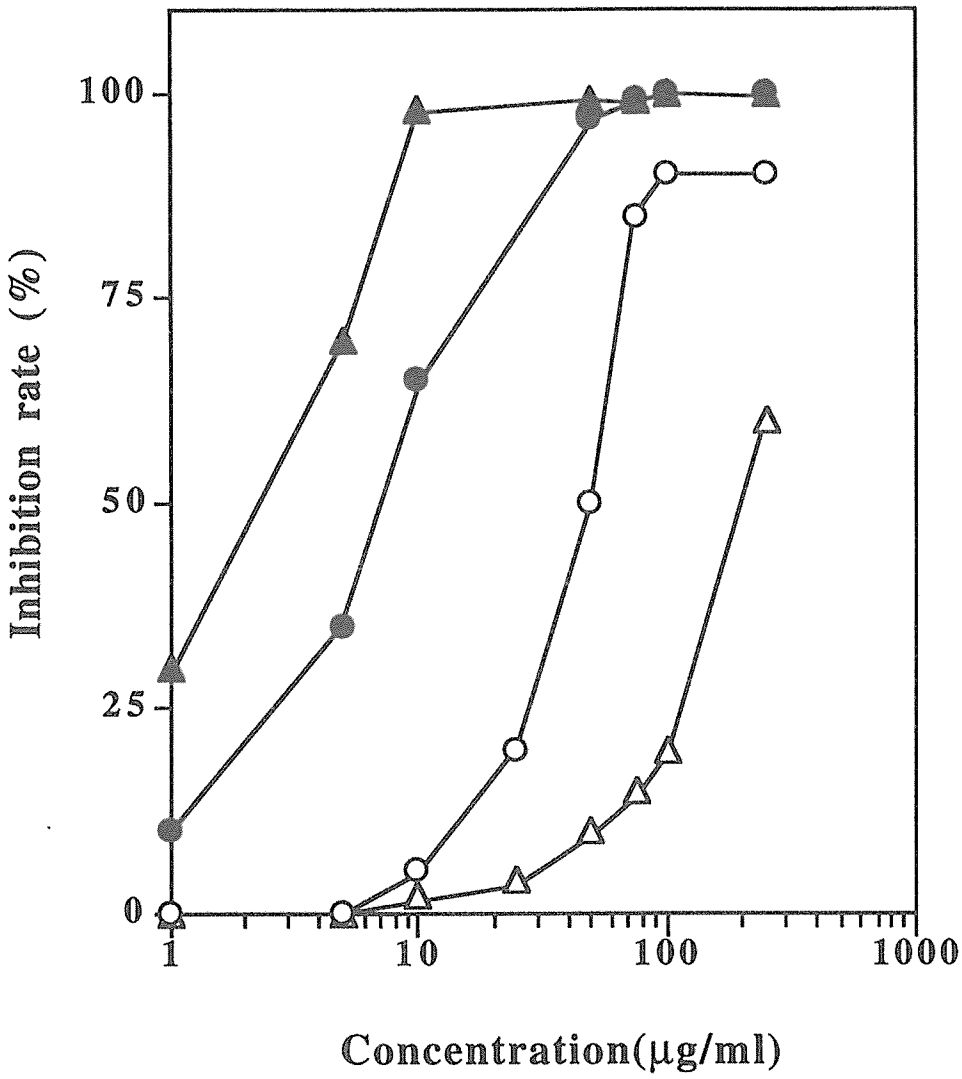


Fig. 28 GTase inhibitory activity of 70 % aceton extracts from several sources as a function of their concentrations.

Legend : ▲ Karamatsu ● Acacia
○ Oolong tea △ Green tea

Table 10 Flavanol values of bark and oolong tea extracts.

Sample	Flavanol value (%)
Oolong tea	5
Acacia	43
Karamatsu	60

Flavanol value (%) was calculated by the calibration using (+)-catechin in the vanillin-hydrochloric acid method.

oolong tea extracts. Table 10 shows the flavanol values of bark and oolong tea extracts measured by the vanillin-hydrochloric acid method. Since these values correlate to the contents of polyphenols, the GTase inhibitory activity of these extracts was presumed to depend on the polyphenols content.

Relationship between the inhibitory activity and the structure of polyphenols

The molecular weight of the each eluate fraction prepared according to Fig. 20, and their IC_{50} values on GTase are shown in Table 11. The molecular weight of these fractions increased in the following order, EE, ME, and AE fraction. Their IC_{50} values decreased with the increase of the molecular weight, which means that the inhibitory activity depends on the molecular weight of polyphenols as shown in Fig. 29. The amounts of nuclei liberated by NER of the natural polyphenol fractions are shown in Table 12. As described in chapter II, NER is an effective method to know about the hydroxylation patterns of A- and B-ring constituting condensed tannins. In acacia extracts, phloroglucinol and resorcinol nuclei were liberated in the ratio of about 1 : 3 from A-ring, catechol and pyrogallol nuclei in the ratio of about 2 : 3 from B-ring. These ratios indicate that acacia polyphenols are of mainly profisetinidin and prorobinetinidin types. On the other hand, the polyphenols in karamatsu consists mainly of procyanidin type as can be seen in most of coniferous bark polyphenols, further quebracho polyphenols consist mainly of profisetinidin type. From the IC_{50} values of ME fractions in different species, which have almost the same molecular weights, the inhibition effect of K-ME is the largest, and those of A-ME

Table 11 The influences of molecular weight of fractionated bark extracts on GTase activity.

Sample	$\overline{Mn}$	$\overline{Mw}$	Dpw	IC50 (μg/ml)
A-EE	355	720	2.4	250
A-ME	1045	1495	3.9	15.0
A-AE	2474	4235	11.4	1.7
K-EE	325	750	2.2	13.0
K-ME	1150	1585	4.0	5.0
K-AE	2852	4825	13.5	1.0

Dpw: Degree of polymerization

IC50 : The concentration (μg/ml) giving 50% inhibition of GTase.

Sample: refer to Fig. 20.

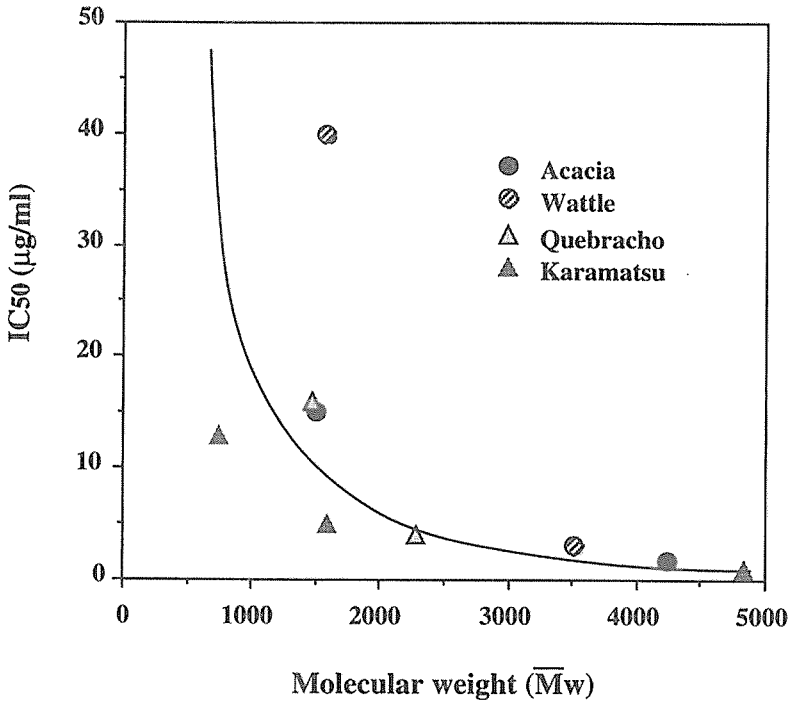


Fig. 29 The relationship between molecular weight of condensed tannins and the GTase inhibitory activity.

IC50: See Table 11

Table 12 Phenol nucleus composition of natural condensed tannins.

(mol %) *

Sample	A-ring		B-ring		Phl./Res.	Pyr./Cat.
	Phl.	Res.	Cat.	Pyr.		
A-ME	15.1	49.5	22.1	38.1	0.31	1.7
A-AE	11.3	33.1	17.3	35.6	0.34	2.1
W-ME	15.7	57.7	24.4	37.3	0.27	1.5
W-AE	15.8	47.3	30.0	42.0	0.33	1.4
Q-ME	5.3	40.6	64.4	2.0	0.13	0.03
Q-AE	3.3	43.3	63.6	2.3	0.08	0.04
K-EE	9.4	0.6	43.8	2.2	15.6	0.05
K-ME	20.5	0.5	65.0	2.8	41.0	0.04
K-AE	23.4	0.6	70.3	3.1	39.0	0.04

Sample name: refer to Fig. 20.

*** These values are represented by mol % to a unit of flavan-3-ol.**

Phl.: phloroglucinol **Res.: resorcinol**

Cat.: catechol **Pyr.: pyrogallol**

and Q-ME are almost the same. Therefore, it is presumed that the A-ring influenced the inhibition of GTase rather than the B-ring did, in particular, phloroglucinolic A-ring was more effective than resorcinolic one. This result is different from the idea that the hydroxyl groups in B-ring rather than those in A-ring form hydrogen bond with proline in the haze forming protein to produce the polyphenol-protein complex.[100] Kawamoto and others,[101] however, reported that hydroxyl groups in A-ring influenced in a similar manner as that in B-ring on the precipitation forming ability with BSA by using some chemically synthesized condensed tannins having several hydroxylation patterns. Therefore, the inhibition mechanism of GTase with condensed tannins may be the same as that of the BSA precipitation.

Table 13 The inhibitory effects of synthesized polyphenols on the GTase activity.

Sample	Concetration (μg/ml)						IC_{50} (μg/ml)
	300	100	30	10	3	1	
Catechin	13	2	1	1	0	0	>300
PF-dimer	29	11	11	10	10	2	>300
PC-dimer	35	13	10	10	10	5	>300
Compound I	75	37	20	15	10	5	153
Compound II	30	15	10	5	2	0	>300
Compound III	35	25	15	10	2	0	>300

IC_{50} : see Table 11.

The influences of pyran-ring opened structure of polyphenols on inhibitory activity

IC_{50} of (+)-catechin, PC dimer, PF dimer, Compound I, II, and III in the inhibitory activity on GTase are shown in Table 13. These low molecular weight polyphenols show only slight inhibitions compared with the natural polyphenols. This result agrees with an observation that a class of monomeric and dimeric polyphenols had no ability to precipitate a protein, and the ability begun to appear from a trimeric polyphenol.[102] However, Compound I showed less than a half of procyanidin dimer in IC_{50} value in spite of its lower molecular weight than the dimer's. Compound I also showed a smaller IC_{50} value than any other phenolated compounds, Compound II and III.

Table 14 shows IC_{50} value, degree of pyran-ring opening (DPO), and molecular weight of phenolated polyphenols prepared from K-ME and A-ME. For the phenolated K-ME, the IC_{50} value did not change largely during the phenolation, though the molecular weight decreased with the increase of phenolation time. On the other hand, the molecular weight of phenolated A-ME nevertheless did not decrease largely, their IC_{50} value rapidly increased.

Table 14 GTase inhibitory effects of the phenolation products from natural condensed tannins.

Sample	Phenolation time (min.)	$\overline{M}w$	DPO	IC50 (μg/ml)
K-ME	0	1585	0	5
	30	982	33.3	7
	120	775	35.5	7
	240	523	40.0	36
A-ME	0	1495	5.2	15
	30	1235	12.3	53
	120	1220	15.7	235
	240	1115	15.2	255

DPO: see Table 6.

IC50: see Table 11.

These phenomena probably relate to the DPO value of the phenolated poplyphenols. That is to say, it is expected that the GTase inhibitory activity of the polyphenols increases by the modification including the pyran-ring opening to produce a flexible structure which may have a high affinity to the enzymes in spite of the decrease of their molecular weight during the phenolation.

III-2-4 Summary

The inhibitory effect of polyphenols with the opened pyran-ring on GTase relating to dental caries was examined. It is well known that green tea or oolong tea have an effective inhibition to GTase. All polyphenols extracted from some barks were more effective inhibitors than that from the teas. The inhibitory activity of bark polyphenols increased with the increase of their molecular weight, and the oxygenation patterns of A-ring in polyphenols more strongly affected the inhibitory activity than that of B-ring. Compound I, pyran-ring opened compound produced from (+)-catechin by the treatment with BF_3 and excess phenol, displayed stronger inhibitory effect than synthesized proanthocyanidin dimers. Further, IC_{50} value of pyran-ring opened and phenolated products originated from K-ME did not increased largely in spite of decreasing of the molecular weight.

Therefore, the flexible structure given by opening the pyran-ring of the poyphenols would play an important role in the GTase inhibition.

CONCLUSION

Present-day exhaust mass of a bark in Japan reaches 5.6 million tons per year. Most of them are not utilized and are regarded as troublesome products in lumber industry. It is however needless to say that bark is very important tissue in the meaning of protecting a tree from external causes. Actually, condensed tannins being contained highly in bark possess functions such as protein precipitation, metal chelation, and nucleophilicity. Nevertheless, a polyphenol industry does not exist in Japan, only a small amount of hydrolyzable tannins is used to remove proteins in the brewery industry.[100,103-105] On the other hand, the development of wood adhesives using condensed tannins has been advanced actively in South Africa, North America, and Australia. However, a utilization as substitutive materials for synthesized phenols such as resorcinol and phlorogrucinol is merely proposed.

The author pointed out the structural problem of condensed tannins, that is the low flexibility on the molecules. Therefore, the molecular denaturation relating to the pyran-ring opening of condensed tannins to increase the flexibility of the molecule was demonstrated in this thesis. The mechanisms of pyran-ring opening and the characteristics of the products of the phenolation of condensed tannins by using BF_3-phenol system are concluded as follows.

In chapter Ⅰ, the phenolation of (+)-catechin which is one of the terminal unit of condensed tannins was performed at 40℃. Three phenol adducts were mainly produced in high yields, especially the yield of Compound Ⅰ was about 60mol %. Opening of the pyran-ring was confirmed on Compound Ⅰ from its 2D-NMR data. That is to say, the phenolation using BF_3 is a selective reaction for opening of the pyran-ring of (+)-catechin. Furthermore, a high accessibility of BF_3 to the substrate and a large yield of Compound Ⅰ in the aprotic solvents were confirmed from the results of the ctalytic activities and the solvent effects. It was proved that the pyran-ring opening reaction proceeded via S_N2 reaction with a phenol at C-2 position of (+)-catechin based on the stereochemistry of Compound Ⅰ.

In chapter Ⅱ, M-NER was deviced as an analytical method to determine a frequency of pyran-ring opening in the phenolation products. To establish this method, synthesized condensed tannins were used. Compared with the NER, fairly large amounts of nuclei were liberated from A-ring by the M-NER. On applying this method to the several natural condensed tannins, pyran-ring opening was recognized in some materials, and surprisingly 60% of the total pyran-ring were estimated to open in quebracho extracts. Since there have been no reports relating to the frequency of pyran-ring opening so far, therefore, the

M-NER method would become one of the effective analysis tool to obtain the information on pyran-ring opening of natural condensed tannins.

In chapter III, the characteristics of phenolated products of bark extracts by BF_3-phenol system were described.

First, the reactivities of the products with formaldehyde which is an important cross-linking reagent for wood adhesives were examined, and the results are summarized as follows: 1) Opening of the pyran-ring accompaning decrease in the molecular weights of condensed tannins was caused by phenolation using inexpensive phenol in the presence of BF_3. A unit which possesses a phloroglucinolic A-ring opened the pyran-ring and cleaved interflavanoid bond more easily than did a unit which possesses a resorcinolic A-ring. 2) The phenolated products of karamatsu extracts accelerated the condensation with formaldehyde in comparison with karamatsu extracts. Therefore, it was indicated that the pyran-ring opening and interflavanoid bond cleaving of the condensed tannins would lead their molecule to the structures increasing basicity and flexibility.

Second, the inhibitory activity of phenolated condensed tannins on glucosyltranferase (GTase) with regard to dental caries was examined. All bark extracts used showed high inhibitory activities, and are recognized to have sufficient functions as priventors of dental caries. However, the inhibition rate decreased with a decrease of molecular weight of natural condensed tannins. (+)-Catechin and synthesized dimer had little or no effect on the inhibition. On the other hand, Compound I and phenolated products of the bark extracts in which they have a pyran-ring opened structure showed high inhibitions in spite of having low molecular weight. That is to say, an importance of the molecular flexibility of condensed tannins were noted in the GTase inhibition mechanisms.

Therefore, modificaion to open the pyran-ring and diminish the molecular weight of condensed tannins were achieved by the phenolation using BF_3, and it is concluded that condensed tannins were converted into more active molecules in chemical and biological points.

REFERENCES

1) Feeny, P. P. : *Phytochemistry*, 8, 2119-2125 (1969)

2) Feeny, P. P. : *Ecology*, 51, 565-573 (1970)

3) Scalbert, A. and Haslam, E. : *Phytochemistry*, 26, 3191-3195 (1987)

4) Viviers, P. M. :*J. Chem. Soc. Perkin Trans., I*, 17-22 (1983)

5) Roux, D. G. : *Phytochemistry*, 11, 1219-1225 (1972)

6) Karchesy, J. J. and Hemingway, R. W. :*J. Agric. Food. Chem.*, 28, 222-228 (1980)

7) Czochanska, Z., Foo, L. Y., Newman, R. H. and Portor, L. J. : *J. Chem. Soc. Chem. Commun.*, 375-379 (1979)

8) Porter, L. J. : *New Zealand Journal of Science*, 17, 213-218 (1974)

9) Yazaki, Y. and Hillis, W. E. : *Holzforschung*, 31, 20-25 (1977)

10) Haslam, E.: "Plant Polyphenols", Cambridge University Press, 1989, P.9-13.

11) Samejima, M. and Yoshimoto, T. : *Mokuzai Gakkaishi*, 25, 671-677 (1979)

12) Ohara, S., Hemingway,R.W. : *Holzforschung*, 43, 149-154 (1989)

13) Hemingway, R. W. and Karchesy, J. J.: "Chemistry and Significance of Condensed Tannins", Plenum Press, 1989, p.3-20.

14) Rosenheim, O. : *Biochem. J.*, 14, 178 (1920)

15) Bate-Smith, E. C. and Swein, T.,: "Comparative Biochemistry", Academic Press, 1962, p.764-775.

16) Porter, L. J.: "Flavans and proanthocyanidins", Chapman and Hall Press, 1988, 23-25.

17) Middlekoop, T. B. and Labadie, R. P. : *de. Wilde. Z. Naturforsch*, 40B, 855-890 (1985)

18) Weinges, K. and Goritz, K. : *Leibigs Ann. Chem.*, 715, 164-175 (1968)

19) Roux, D. G. and Ferreira, D. : Fortschr. Chem. Org. Naturst., 41, 48-54 (1982)

20) Roux, D. G. and Ferreira, D. : Rev. Pure and Applied Chem., 54, 2465-2469(1982)

21) Porter, L. J., Hrstich, L. N. and Chan, B. G. : *Phytochemistry*, 25, 223-228 (1986)

22) Betts, M. J., Brown, B. R., Brown, P. E. and Pike, E. T. : *J. Chem. Soc. Chem. Commun.*, 1110-1118 (1967)

23) Betts, M. J., Brown, B. R. and Shaw, M. R. : *J. Chem. Soc. Chem. Commun.*, 1178-1183 (1969)

24) Brown, B. R. and Shaw, M. R. : *J. Chem. Soc. Perkin Trans. I*, 2036-2042 (1974)

25) Kennedy, J. A. and Powell, H. K. J. : *Aust. J. Chem.*, 38, 879-889 (1985)

26) Takechi, M., Tanaka, Y., Takehara, M., Nonaka, G. and Nishioka, I. : *Phytochemistry*,

24, 2245-2249 (1985)
27) Matsuyama, M., Kuratani, Y. and Ito, T. : *Mokuzai Kogyou*, 38, 492-496 (1983)
28) Pizzi, A. : *Holz als Roh- und Werkstoff*, 40, 293-301 (1982)
29) Pizzi, A. : *Forest Prod. J.*, 28, 42-47 (1978)
30) Kreibich, R. E. and Hemingway, R. W. : *Forest Prod. J.*, 37, 43-46 (1987)
31) Haslam, E. : *Phytochemistry*, 16, 1625-1640 (1977)
32) Pizzi, A. : *Colloid and Polymer Sci.*, 257, 37-40 (1979)
33) Foo, L. Y., McGraw, G. W. and Hemingway, R. W. : *J.Chem.Soc. Chem. Commun.*, 672-673 (1983)
34) Herrik, F. W. : *J.Agric.Food.Chem.*, 28, 228-237 (1980)
35) Hemingway, R. W., Karchesy, J. J., McGraw, G. W. and Wielesek, R. A. : *Phytochemistry*, 22, 275-281 (1983)
36) Hemingway, R. W., Foo, L. Y. and Porter, L. J. : *J.Chem.Soc. Perkin Trans. I*, 1209-1216 (1982)
37) Brandon, M. J., Foo, L. Y., Porter, L. J. and Meredith, P. : *Phytochemistry*, 21, 2953-2957 (1982)
38) Sun, D., Wong, H. and Foo, L. Y. : *Phytochemistry*, 26, 1825-1829 (1987)
39) Kreibich, R. E. and Hemingway, R. W. : *Forest Prod.J.*, 35, 23-25 (1985)
40) Roùx, D. G., Ferreira, D., Hundt, H. K. and Malan, E. : *J. Appl. Polym. Sci.*, 28, 335-340 (1976)
41) Pizzi, A. and Roux, D. G. : *J. Appl. Polym. Sci.*, 22, 2717-2718 (1978)
42) Roux, D. G., Ferreira,D., Botha,J.J. : *J. Agric. Food. Chem.*, 28, 216-222 (1980)
43) Hemingway, R. W. and Kreibich, R. E., : *J. Appl. Polym. Sci. Appl. Polym. Sympo.* 40, 79-90 (1984)
44) Sasaya, T., Ozawa, S. and Kodama, Y. : *Proceedings of The Hokkaido Branch of The Japan Wood Research Society*, 21, 70-73 (1989)
45) Laks, P. E., Hemingway, R. W. and Conner, A. H. : *J.Chem.Soc. Perkin Trans. I*, 1875-1881 (1987)
46) Abe, I., Funaoka, M., Kodama,M. : *Mokuzai Gakkaishi*, 33, 582-588 (1987)
47) Sears, K. D. : *J. Org. Chem.*, 37, 3546 (1972)
48) Rahman, A. U.: "Saishin NMR (The Newest NMR)", Spring-Verlag Tokyo, 1988, p.60-70.
49) Hemingway, R. W. and McGraw, G. W. : *J.Wood Chem. Tech.*, 3, 421-435 (1983)
50) Hemingway, R. W. and Daling, G. M. E. : *J. Appl. Polym. Sci.*, 25, 1039-1048 (1980)
51) Ohara, S. and Hemingway, R. W. : *J. Wood Chem. and Technol.*, 11, 195-208 (1991)
52) Foo, L. Y. and Porter, L. J. : *J.Chem.Soc. Perkin Trans. I*, 1535-1543 (1983)

53) Morison, R. T. and Boyd, R. N.: "Organic Chemistry", Tokyo Kagaku Doujin, 1989, p.930-940.

54) Ashida, T. et al.: "Kagaku Binran II", Y. Sasada, ed. Maruzen, 1991, p.717-718.

55) Winstein, S., Lindegren, C. R. and Ingraham, L. L. : *J. Am. Chem. Soc.*, 75, 534-550 (1953)

56) Grunwald, S. and Winstein, S. : *J. Am. Chem. Soc.*, 70, 1235-1239 (1953)

57) Gram, D. J. : *J. Am. Chem. Soc.*, 74, 2159-2166 (1952)

58) Nonaka, G., Morimoto, S. and Nishioka, I. : *J. Chem. Soc. Perkin Trans. I*, 2139-2145 (1983)

59) Czochanska, Z., Foo,L.Y., Newman,R.H., Portor,L.J. : *J. Chem. Soc. Perkin Trans. I*, 2278-2286 (1980)

60) Fletcher, A. C., Porter, L. J., Haslam, E. and Gupta, R. K. : *J. Chem. Soc. Perkin Trans. I*, 1628-1637 (1977)

61) Gupta, R. K., Haslam, E. : *J. Chem. Soc. Perkin Trans. I*, 892-896 (1978)

62) Nonaka, G., Hsu, F. and Nishioka, I. : *J. Chem. Soc. Chem.Commun.*, 781-783 (1981)

63) Thompson, R. S., Jacques,D., Haslam,E. : *J. Chem. Soc. Perkin Trans. I*, 1387-1399 (1972)

64) Nonaka, G. and Nishioka, I. : *Chem. Pharm. Bull.*, 30, 4277-4282 (1982)

65) Stafford, H. A., Lester, H. H. and Porter, L. J. : *Phytochemistry*, 24, 333-338 (1985)

66) Chiang, V. L. and Funaoka, M. : *Holzforschung*, 44, 147-155 (1990)

67) Funaoka, M., Abe,I. : *Mokuzai Gakkaishi*, 31, 671-676 (1985)

68) Demachi, H., Terasawa, M., Sasaya, T. : *Proceedings of The Hokkaido Branch of The Japan Wood Research Society*, 1, 57-62 (1968)

69) Kondo, R., Imamura, H. : *Mokuzai Gakkaishi*, 31, 927-934 (1985)

70) Porter, L. J., Foo,L.Y. : *Phytochemistry*, 21, 2947-2952 (1982)

71) Young, D. A., Cronje,A., Botes,A.L., Ferreira,D., Roux,D.G.: *J. Chem. Soc. Perkin Trans. I*, 2521-2527 (1985)

72) Botha, J. J., Ferreira,D., Roux,D.G. : *J. Chem. Soc. Perkin Trans. I*, 1235-1245 (1981)

73) Bate-Smith, E. C. : *Phytochemistry*, 14, 1107-1113 (1975)

74) Takahashi, H., Abe,I., Mitsunaga,T. : *Abstracts of 41 th Anual Meeting of Japan Wood Research Society*, 1991, p.176.

75) Samejima, M., Yoshimoto,T. : *Mokuzai Gakkaishi*, 27, 658-662 (1981)

76) Gupta, R. K., Haslam,E. : *J. Chem. Soc. Perkin Trans. I*, 1148-1150 (1981)

77) Samejima, M., Yoshimoto,T. : *Mokuzai Gakkaishi*, 28, 67-74 (1982)

78) Ohara, S., Suzuki, K. and Ohira, T. : *Mokuzai Gakkaishi*, 40, 1363-1374 (1994)

79) Nonaka, G., Nishioka,I. : *Chem. Pharm. Bull*, 28, 3145-3149 (1980)
80) Mitsunaga, T. and Abe, I. : *Abstracts of the 44th annual meeting of the Japan Wood Research Society*, 1994, p.238.
81) Kiatgrajai, P., Wellons, J. D., Gollob, L. and White, J. D. : *J. Org. Chem.*, 47, 2913-2917 (1982)
82) Kanemaru, K. : "Setchaku to setchakuzai", Dainippon tosho, 1969, p.79-90.
83) Hillis, W. E. and Urbach, G. : *J. Appl. Chem.*, 9, 665-673 (1959)
84) Hemingway, R. W., McGraw,G.W. : *Journal of Liquid Chromatography*, 1, 163-179 (1978)
85) Foo, L. Y., Hemingway,R.W. : *J. Wood Chem. Tech.*, 5, 135-158 (1985)
86) Jones, R. T. : *Journal Polymer Science*, 21, 1801-1817 (1983)
87) Pizzi, A. : *J. Macromol. Sci., Rev. Macromol. Chem.*, C2, 247-315 (1980)
88) Takano, R. : *J.The Toyama Forestry and Forest Products Research Center*, 6, 50-58 (1992)
89) Yoshimoto, T.: "Ki no hanashi", Ootsuki shoten, 1983, p.210-212.
90) Mole, S. and Waterman, P. G. : *Phytochemistry*, 26, 99-104 (1986)
91) Hall, C. B. : *Nature*, 212, 717-719 (1966)
92) Goldstein, J. L. and Swein, T. : Phytochemistry, 4, 185-193 (1965)
93) Inokuchi, J., Okabe, H., T., Y., Nagamatsu, A., Nonaka, G. and Nishioka, I. : *Chem. Pharm. Bull.*, 33, 264-269 (1985)
94) Wu-yuan, C. D., Chen, C. Y. and Wu, R. T. : *J. Dent. Res.*, 67, 51-55 (1988)
95) Kakiuchi, N., Hattori, M. and Nishizawa, M. : *Chem. Pharm. Bull.*, 34, 720-725 (1986)
96) Nakahara, K., Kawabata, S., Ono, H., Ogura, K., Tanaka, T., Ooshima, T. and Hamada, S. : *Applied and Enviornmental Microbiology*, 968-973 (1993)
97) Ooshima, T., Minami, T. and Hamada, S. : *Caries Res.*, 27, 124-129 (1993)
98) Sakanaka, S., Kim, M., Taniguchi, M. and Yamamoto, T. : *Agric. Biol. Chem.*, 53, 2307-2311 (1989)
99) Sakanaka, S. : *Agric. Biol. Chem.*, 54, 2925-2929 (1990)
100) Asano, K., Hashimoto,N. : *J. Am. Soc. Brewing Chemists*, 40, 147-154 (1982)
101) Kawamoto, H., Nakatubo, F. and Murakami, K. : *Mokuzai Gakkaishi*, 37, 741-747 (1991)
102) Kawamoto, H., Nakatsubo, F. and Murakami, K. : *J. Wood Chem. Technol.*, 10, 401-409 (1990)
103) Asano, K., Ohtsu, K., Shinagawa, K. : *Agric. Biol. Chem.*, 48, 1139-1146 (1984)
104) Chibata, I., Tosa, T., Mori, T., Watanabe, T. and Sakata, N. : *Enzyme Microb. Technol.*, 8, 130-136 (1986)
105) Watanabe, T., Mori, T. and Sakata, N. : *Hakkokogaku*, 57, 141-147 (1979)

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