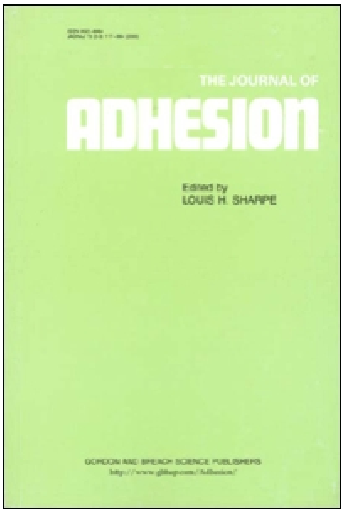

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Reaction of Catechin and Tannin with *N*-Hydroxymethylacrylamide

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Reaction of Catechin and Tannin with *N*-Hydroxymethylacrylamide

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For the purpose of understanding the mechanism of the reaction between tannin and poly(N-hydroxymethylacrylamide) (pNMA) reported in the preceding paper, corresponding elemental reactions of catechin and tannin with N-(hydroxymethyl)acrylamide monomer (NMA) were studied. NMA reacted with both catechin and tannin under acidic conditions, and analyses of the isolated products by NMR and mass spectra revealed the chemical structures of the adducts, supporting the effective adhesion shown in the preceding work. The evidence indicating the decreased solubility of the adduct in water explains the enhanced water resistance of bonding by the tannin-pNMA adhesive.

Keywords: Adduct; Catechin; MALDI; NMA; NMR; Tannin

INTRODUCTION

Since an adhesive based on the reaction of tannin with poly(*N*-hydroxymethylacrylamide) (pNMA) gave water resistant bonding of wood as shown in the preceding paper [1], corresponding elemental reactions of catechin and tannin with *N*-(hydroxymethyl)acrylamide monomer (NMA) were studied in order to understand the mechanism of adhesive bonding. Although there were reports on the reaction of catechin with formaldehyde [2], hydroxybenzyl alcohols [3], and *N*-hydroxymethylacetoamide [4], the reaction with NMA was unprecedented. NMA reacted with both catechin and tannin,

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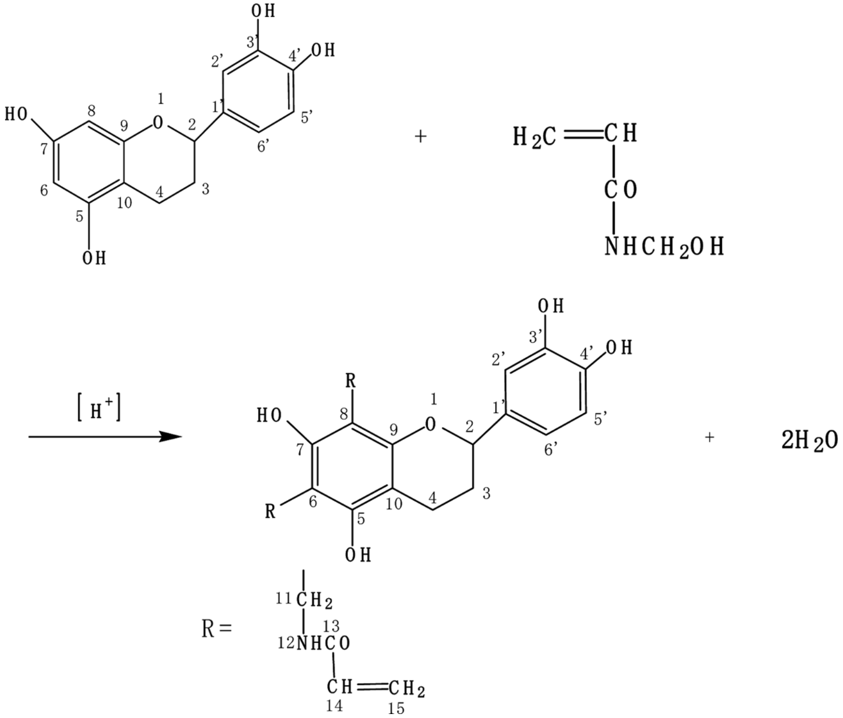

FIGURE 1 Postulated reaction sequence of catechin with NMA.

and analyses of isolated products by NMR and mass spectroscopy revealed the chemical structures of the condensation products. Thus, this report presents a chemical background of wood bonding with an adhesive consisting of tannin and pNMA (Figure 1).

EXPERIMENTAL

Materials and Instruments

Mimosa tannin, Plytan-AP, a commercial product of Oshika Co., Tokyo, Japan and reagent grade catechin (98% pure, Sigma-Aldrich, Tokyo, Japan) were used. NMA and other conventional chemicals such as ethanol, chloroform, THF, ethyl acetate, and so on were reagent grade chemicals of Nakarai Tesque Co., Kyoto, Japan. Mass spectra were measured by Tandem MStation of JEOL (JMS-700, Tokyo, Japan) for fast atom bombardment-mass spectroscopy (FAB-MS) and high resolution mass spectrometry (HRMS), and Kompact MALDI

IV of Shimadzu/Kratos, Kyoto, Japan for matrix-assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF-MS). ^{13}C-nuclear magnetic resonance spectra were analyzed by JNM-270EX FT-NMR, 270 MHz, JEOL, Japan. NMR solvents such as methanol-d_4 and DMSO-d_6 were products of Isotec Inc., Sigma-Aldrich, Tokyo, Japan, with 100% and 99.6 atom%-*d*, respectively.

Reaction of Catechin with NMA

In a 200 ml three-neck flask, catechin (0.29 g, 1 mmol), NMA (424 mg, 4.2 mmol), THF (10 ml), and HCl (1 ml of 1 mol/l aqueous solution) were added and stirred for 72 h at room temperature. The reaction mixture was then poured into ethyl acetate and washed with aqueous sodium bicarbonate and brine (three times each), and the products were separated in ethyl acetate. The organic solution was dried with sodium sulfate and, after concentration, the residue was dried under reduced pressure to obtain a yellow solid (451 mg). Thin layer chromatography (TLC) of the yellow product showed two main spots at *Rf* of 0.75 and 0.5 with eluting solvent of 1:3 v/v methanol/chloroform. The fast-eluting product (I, *Rf* = 0.75, 140 mg) was separated from 300 mg of crude yellow solid through a silica gel column. Attempts to isolate the other product were not successful.

The mass spectrum of Adduct I was measured by FAB-MS to obtain peaks at 456 and 457 m/z in a positive mode (Figure 2). By HRMS the (M + 1) ion peak was observed at 457.1619 m/z.

Reaction of Tannin with NMA

Tannin (10.3 g) was added to methanol (70 ml). After stirring the mixture for about 30 min at room temperature, the insoluble fraction in methanol was separated off by filtration. The insoluble brown solid amounted to 1.58 g (15.3%) after drying. In a four-neck 500 ml separable flask equipped with a mechanical stirrer, a dropping funnel, and a condenser, the filtered solution of tannin and NMA (3.06 g) was added, and after adjusting to pH 3 with aqueous HCl, the mixture was warmed up to 60°C, and refluxed for 3 hours under mechanical stirring. The reaction mixture was then concentrated and vacuum dried. The solid reaction product was washed with distilled water to yield a reddish brown solid of NMA-tannin adduct. This reddish brown solid was insoluble in distilled water but highly soluble in acetone. Since tannin is insoluble in acetone but soluble in distilled water, the solubility of the product indicates some reaction of tannin to give products of different physical properties. The product was washed

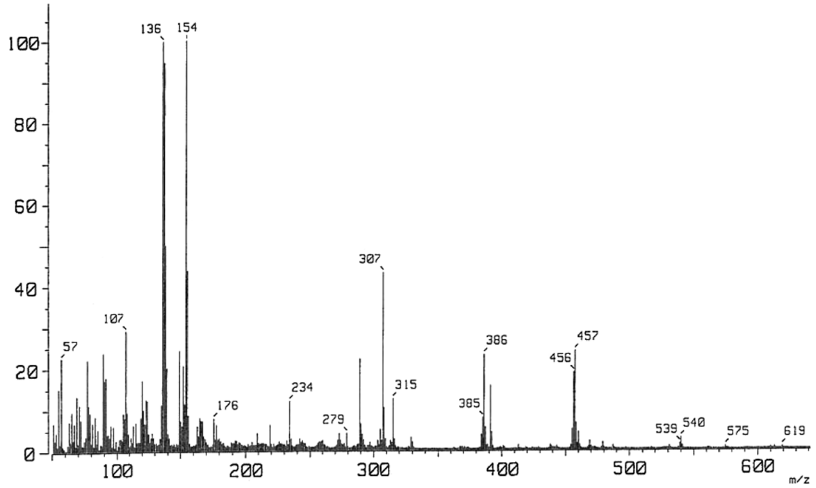

FIGURE 2 FAB-MS of catechin-NMA, Adduct I.

repeatedly with distilled water to give a brown solid of tannin-NMA adduct (II) in a yield of 10.6 g.

MALDI-TOF-MS Spectra of Tannin-NMA Adduct (II)

Analyses were carried out as follows: Tannin was dissolved in methanol (500 mg/l) and filtrated. A 10-μl portion of this solution was mixed with 5 μl of matrix solution (1% dithranol in methanol). A 5 mg sample of tannin-NMA adduct was dissolved in 10 ml acetone, and a 10 μl portion of the adduct solution was mixed with 5 μl of matrix solution (2,5-dihydroxybenzoic acid: sodium iodide = 1:1 in mol ratio). These solutions were put on a target plate, crystallized at room temperature, and subjected to MALDI-TOF-MS analysis. The obtained MS spectra of tannin and tannin-NMA adduct are shown in Figures 3 and 4, respectively. From the MALDI-TOF-MS spectra the most abundant molecular weights of tannin were observed at 1451, 1161, and 871 m/z. On the other hand, the main peaks of NMA-tannin adduct were observed at 1904, 1821, 1738, 1200, 1117, and 1034 m/z.

RESULTS AND DISCUSSION

Characterization of Adduct of Catechin-NMA (I)

Analysis of isolated product I was carried out by mass spectrometry and NMR (^{1}H- and ^{13}C-NMR spectra, DEPT, and C-H and H-H cosy).

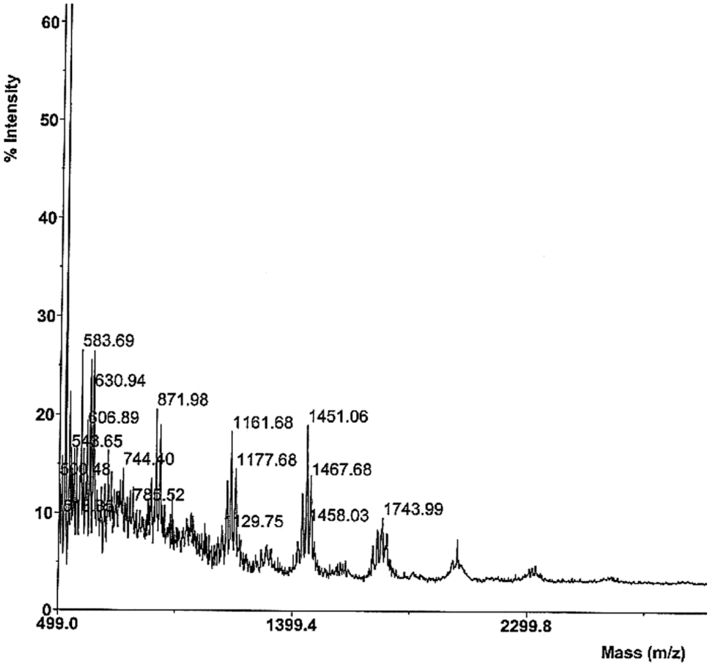

FIGURE 3 MALDI-TOF-MS of tannin.

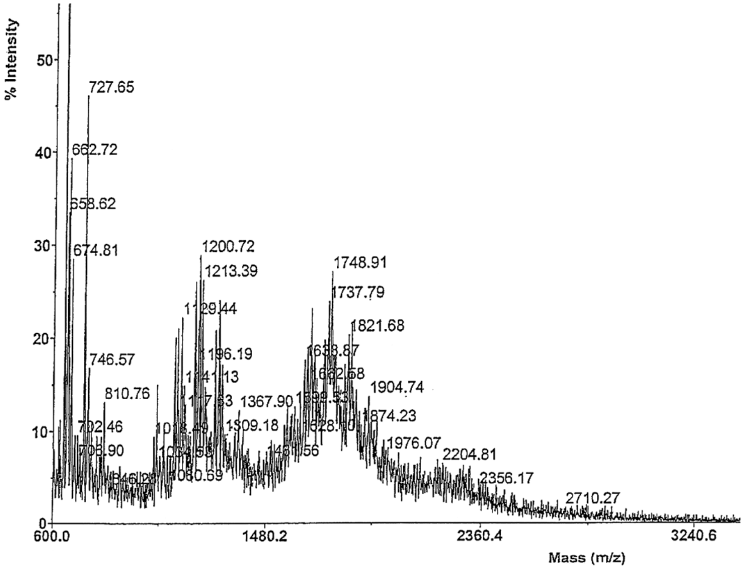

FIGURE 4 MALDI-TOF-MS of tannin-NMA, Adduct II.

TABLE 1 ^{1}HNMR Peak Assignments of Catechin and NMA-Catechin[a]

	Catechin	Catechin-NMA
H-2	4.9d (7.6)	4.8d (6.5)
H-3	4.0m (7.6, 5.4)	4.0m (6.5, 5.5)
H-4	2.8dd (16.3, 5.3) 2.6dd (16.3, 7.9)	2.8dd (16.6, 5.5) 2.6dd (16.6, 6.5)
H-6	5.9d (2.3)	—
H-8	6.0d (2.3)	—
H-2′	6.7m	6.7m
H-5′	6.7–6.8m	6.7–6.8m
H-6′	6.9d (2.0)	6.8d (1.7)
H-11	—	4.4d (10.3)
H-14	—	6.2m
H-15	—	5.7m, 6.2m

[a] Chemical shifts are shown in ppm downward from TMS in methanol-d_4. Values in parentheses show the coupling constants, J, in Hz. Abbreviations: d, doublet; dd, double doublet; m, multiplet.

As described above, the FAB-MS spectrum of Adduct I showed peaks at 456 and 457 m/z in a positive mode (Figure 2). By the high resolution method (HRMS), the $(M+1)$ peak was observed at 457.1619 m/z compared with 457.1566 m/z for the theoretical value of the condensation adduct consisting of catechin and two NMA molecules by removal of two H_2O molecules as shown in the equation (Figure 1).

Results of ^{1}H- and ^{13}C-NMR spectra measured in methanol-d_4 are summarized in Tables 1 and 2, respectively. In the ^{1}H spectra, signals due to H6 and H8 of catechin disappeared and new signals of H11-H15 appeared in the Adduct I (Figure 5). This change of spectrum suggests the reaction of two NMA monomers at the open sites of the aromatic ring of the couromane structure in castachin as shown in the equation.

In the ^{13}C spectra, chemical shifts of C6 and C8 increased after adduct formation corresponding to the substitution. Other chemical shifts of catechin showed negligible or only small changes. New peaks of C11-C15 assigned to NMA residue appeared in the spectrum of Adduct I. The C11 carbon showed a change of chemical shift from NMA monomer (65.4 ppm) to 34.45 ppm for catechin adduct, indicating the methylene group of NMA is the site of reaction. The results of ^{13}C NMR also support the reaction of NMA at the C6 and C8 positions. The structure of Adduct I was further comfirmed by DEPT (Figure 6) and C-H and H-H COSY (Figures 7 and 8).

Thus, by comparison of the corresponding NMR spectrum of Adduct I with those of catechin and NMA, the Adduct I is identified as a condensation product of two NMA methylol groups with 6- and 8-positions

TABLE 2 ^{13}CNMR Peak Assignments of Catechin and NMA-Catechin[a]

	Catechin	Catechin-NMA
C-2	82.7	82.7
C-3	68.7	68.4
C-4	28.3	28.1
C-5	156.8	154.3
C-6	96.3	107.7
C-7	156.8	154.3
C-8	95.5	105.7
C-9	146.1	146.2
C-10	100.8	102.0
C-1′	132.1	132.1
C-2′	115.2	114.9
C-3′	157.4	155.8
C-4′	157.6	155.0
C-5′	116.1	116.2
C-6′	120.0	119.7
C-11	—	34.4
C-13	—	169.6
C-14	—	130.9
C-15	—	127.5

[a] Chemical shifts are shown in ppm downward from TMS in methanol-d_4.

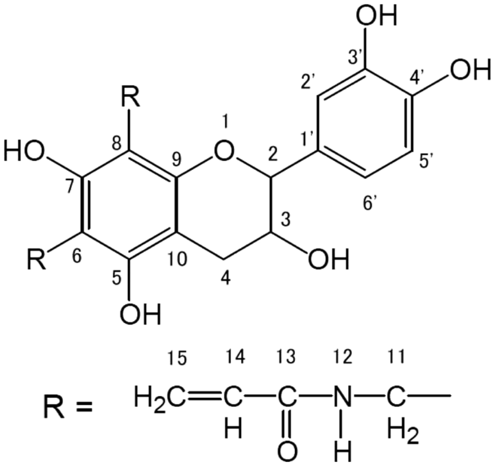

FIGURE 5 Molecular structure of catechin-NMA, Adduct I.

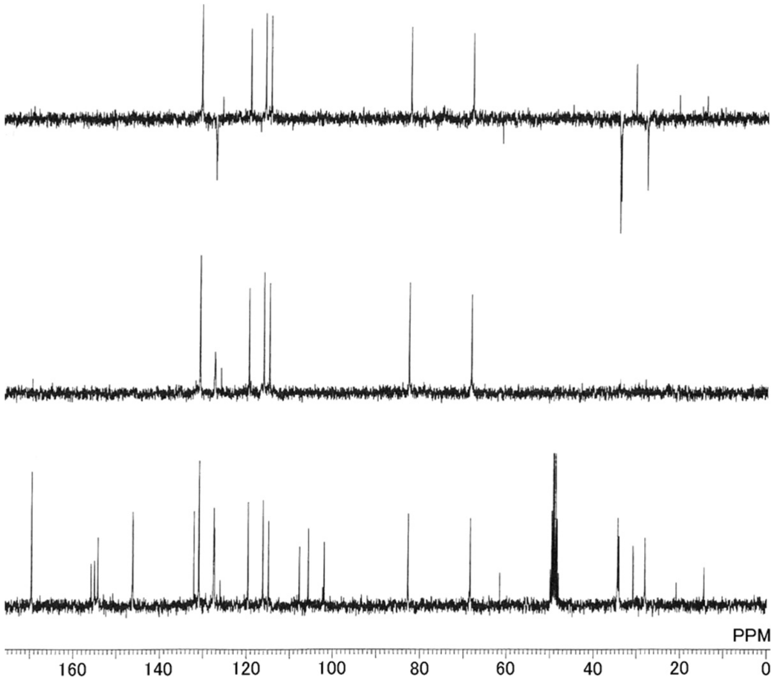

FIGURE 6 DEPT of catechin-NMA.

of catechin to give methylene linkages (Figure 1) as is the case of *N*-hydroxymethylacetamide [4].

Characterization of Adduct of Tannin-NMA (II)

The isolated product of the tannin-NMA reaction, II, was dissolved in DMSO-d_6 and analyzed with ^{13}CNMR and MALDI-TOF-MS. ^{13}CNMR of II showed peaks at 43.4 (a -CH_2O- carbon), 127.6 (an acrylic CH_2= carbon), 131.8 (an acrylic -CH=carbon), and 166.5 (a C=O carbon) ppm from TMS. The changes in the ^{1}H and ^{13}C NMR spectra in the NMA part of Adduct II showed similar tendencies with the reaction of NMA with catechin as described above. Although the peaks due to tannin were not clearly observable with ^{13}CNMR in DMSO-d_6, the NMA part showed clear peaks as shown in Table 3; a hydroxymethylene carbon of the NMA monomer had an absorption at 65.4 ppm in methanol-d_4 and after the reaction with catechin it appeared at 34.5 ppm (in methanol-d_4), while in the tannin adduct the peak appeared at 43.4 ppm (in DMSO-d_6). This change of spectrum and also

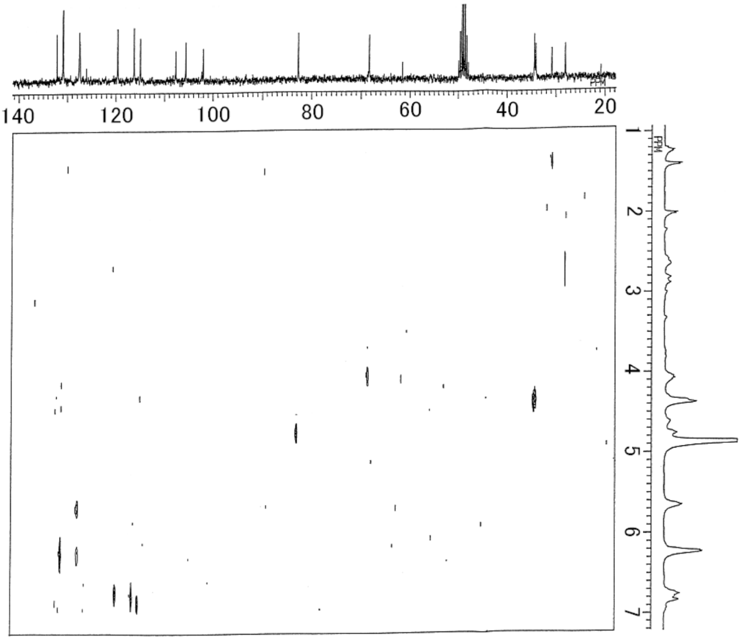

FIGURE 7 C-H cosy of catechin-NMA.

the change in physical property suggest that Product II is a tannin-NMA adduct, which was confirmed by the results of mass analysis.

In the above results of the MALDI-TOF-MS analysis of tannin described in the experimental section, the main molecular weights are observed with a constant interval of 290 m/z (Figure 3). The interval of 290 m/z corresponds to the molecular weight of catechin, revealing that the polymerization of catechin constitutes mimosa tannin [2,5]. In addition, it is notable that the maximum molecular weight of the detectable peak is less than 3000 m/z.

In the MS of the tannin-NMA adduct, peaks are observed with an interval of 83 m/z (Figure 4). The molecular weight of 83 m/z corresponds to the MS difference between the tannin adducts of one NMA and two NMA. Furthermore, the observed peaks can be assigned clearly as the NMA derivatives of catechin as follows: 663 m/z coincides with the total mass of condensation product of one NMA and two catechin units, and 746 m/z corresponds to two NMA and two catechin molecules. Similarly, 1034 m/z corresponds to two NMA and three units of catechin (1036), 1117 m/z to three NMA and three units of catechin (1119), 1200 m/z to four NMA and three units of catechin (1202). The sites of reaction of NMA should be open sites of

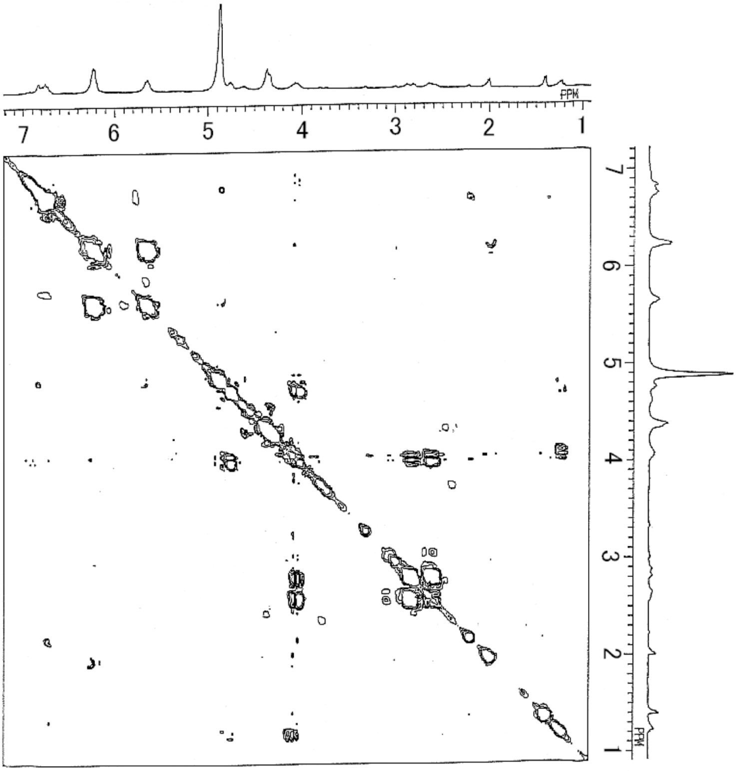

FIGURE 8 H-H cosy of catechin-NMA.

catechin such as the 6 and 8 positions on the basis of the above results on the reaction with catechin. However, possibilities of reactions at other sites of the catechin structure such as the catechol ring cannot be eliminated under strong reaction conditions.

TABLE 3 ^{13}CNMR Peak Assignments of NMA and NMA-Tannin[a]

	NMA	Tannin-NMA
C-1	65.4	43.4
C-3	166.5	165.0
C-4	131.8	131.3
C-5	127.6	125.9

[a] Chemical shifts are shown in ppm downward from TMS in methanol-d_4.

CONCLUSION

We can conclude that evidence for the acid-catalyzed reaction of catechin unit with methylol group of NMA is presented, and the decreased solubility of the Adduct II in water explains the enhanced water resistance of the tannin-pNMA adhesive through bonding.

ACKNOWLEDGMENT

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