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Radical Sulfonation of Condensed Tannins*1

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縮合탄닌의 래디칼 설폰化*1

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摘 要

^{1}H 및 ^{13}C 核磁氣 共鳴 分光器, 赤外線 吸光 分光器와 質量分析器를 利用하여 설폰化 反應의 한 反應物이 單離되어 構造的으로 糾明되었다. 그 化合物은 에탄올 및 에탄올 水溶液을 溶媒로 使用하여 Sephadex LH－20上에서의 反復的인 컬럼 크로마토그래피로 단리 및 精製되었다. 규명된 화합물은 이전에 報告된 바 없는 새로운 구조의 disodium epicatechin－(4β, 5′)－disulfonate였다. 이 화합물은 温和한 설폰化 條件下에서 最初의 親電子 置換의 例로서 catechol B 環構造가 quinone methide 中間物을 生成하는 한 反應機構가 提示되었다.

1. INTRODUCTION

Of particular importance to the forest products industry are the petrochemically derived adhesive resins used to make exterior quality durable bonds in structural wood composite materials(9).

Phenol-formaldehyde (PF) and phenol-resorcinol-formaldehyde(PRF) are presently the adhesives of choice. Also of particular concern to the industry is the continuing availability of PF and PRF resins in the future.

Petroleum is a non-renewable resource and sudden interruptions in its supply can have dramatic effects as evidenced by the world situation in 1973.

Presently there is a great deal of interest in the utilization of condensed tannins in wood adhesive formations in the countries such as New Zealand, Republic of South Africa and the United States.

Therefore, utilization of the polyphenols (condensed tannins) in Douglas-fir bark to replace or supplement phenol and resorcinol in PF and PRF resins would allow the industry to better control its adhesive supply.

It thus becomes apparent that the polymeric condensed tannins of Douglas-fir have the potential to not only replace phenol-but also resorcinol-in the production of phenolic adhesives.

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However, there are also problems to overcome in the utilization of polymeric condensed tannins as adhesive materials. One is the inactivation of the phloroglucinol ring under conditions of high pH to form catechinic acid moieties(12).

This problem can be addressed by using lower pH(8 or below) to achieve polymerization and/or cross linking for curing(5). Another problem is the poor water solubility and high viscosity of condensed tannin adhesive formations.

There would also be an advantage using flavanoid monomers to produce the adhesive polymers rather than the condensed tannin polymers themselves.

Sulfonation of the polymeric condensed tannin offers considerable hope in addressing these later problems. The sulfonation process has been shown to increase water solubility and produce lower molecular weight sulfonated derivatives including monomer sulfonates(3).

However, little attention has been devoted to understanding this sulfonation reaction. This research was to isolate and characterize the sulfonation reaction products of Douglas-fir bark polymeric condensed tannins.

2. EXPERIMENTAL METHOD

2.1. General

^{1}H and ^{13}C nuclear magnetic resonance spectrometry(NMR), fast atom bombardment mass spectrometry (FAB−MS), infra red spectrometry (IR) were used to determine the structure of the sulfonation reaction product.

The ^{13}C and ^{1}H NMR spectra were obtained from a Bruker AM 400 spectrometer with samples dissolved in methanol-d4 and FAB-MS was operated in the negative ion mode on a Kratos MS-50 TC mass spectrometer with samples dissolved in a liquid matrix of a 5 : 1 mixture of dithiothreitol and dithioerythritol(Magic Bullet).

IR spectrum was recorded on KBr pellets using a Nicolet 5DXB FT-IR spectrometer.

Preparative and analytical TLC were performed using Schleicher & Schuell cellulose coated plates, developed with HOAc-H_2O(3 : 47 v/v) and visualized with vanillin-HCl-95% ethanol(600 : 1.5 : 60 w/v) spray.

2.2. Sample preparation and purification

The inner bark used in this study was taken from a freshly fallen 120 year old Douglas-fir [*pseudotsuga menziesii* (mirb.) Franco] tree in McDonald Forest, Benton County, Oregon in May of 1986. The sample extraction and purification were treated according to Bae's procedure(1).

2.3. Sulfonation reaction

A portion of the polymeric condensed tannin(5.0 g) was mixed with sodium hydrogen sulfite(1.75 g) and water(7.5mℓ) in a pressure tube.

After thoroughly stirring, this mixture was perged with nitrogen gas for 10 minutes to remove oxygen from the solution.

The tube was then sealed and put into a steam bath at 105℃ for the reaction. The pH of the solution cheeked by a corning pH/ion meter(model 150) was 4.78 and the reaction time was 36 hours.

2.4. Isolation and purification of condensed tannin sulfonate

The reaction product, after the sulfonation reaction, was freeze dried and then extracted with methanol to remove the condensed tannin sulfonate derivatives from unreacted sodium hydrogen sulfite.

The methanol soluble fraction was filtered and then concentrated on a rotary evaporator to give 5.48 g of crude condensed tannin sulfonate derivatives.

After freeze drying, the sulfonates were then dissolved in water and extracted with ethyl acetate to remove the unreacted free phenolic compounds.

The residual ethyl acetate remaining in the water soluble fraction was evaporated and the aqueous phase then freeze dried.

A portion of the freeze dried powder(4 g) was applied to a Sephadex LH-20 column(6×45cm) for further purification

The column was washed with water at a flow rate of 1mℓ/min. until the eluting solvent was almost colorless.

Fractions were collected by Gilson micro fractionators(FC-100 and model 203). To apply solvent to the column, a FMI Lab pump(model RP-G 50) and FMI pulse dampener(model PD-60-LF) were used.

The eluting sulfonates were detected by a Gilson UV detector(model 111B), its sensitivity was adjusted at 0.5 AUFS and it was operated at 280nm. The chart speed of the recorder(Gilson linear 1200) was 2cm/hr.

Five major peaks were collected and labeled fraction Ⅰ, Ⅱ, Ⅲ, Ⅳ, and Ⅴ. Fraction I was first eluted and Ⅴ was the last.

Each fraction was evaporated under reduced pressure and then freeze dried. The overall purification scheme is shown in Figure 1.

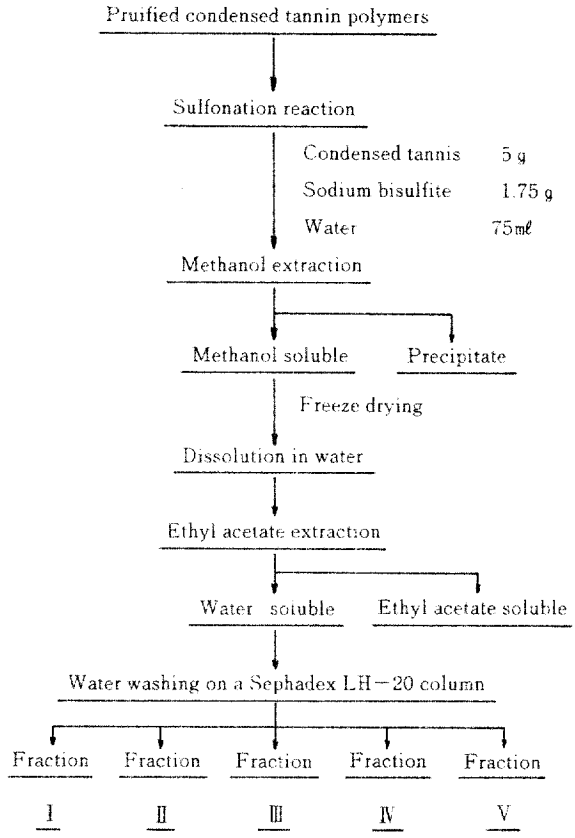


Fig. 1. Purification scheme of procyanidin sulfonates.

2.5. Characterization of condensed tannin sulfonate - disodium epicatechin-(4β, 5')-disulfonate.

Fraction Ⅴ(120mg) was chromatographed on a Sephadex LH-20 column(1.5×65cm) using 95% ethanol as the eluting solvent with a flow rate of 1mℓ/min.

Three fractions were made based on TLC analysis. The first and the second fraction were stocked for further analysis.

The last coming fraction was collected and freeze dried. This fraction exhibited two spots on TLC.

After freeze drying, this fraction was redissolved in ethanol and applied to a Sephadex

LH-20 which was eluted with 95% ethanol-water(7 : 3 v/v) at a flow rate of 1mℓ/min.

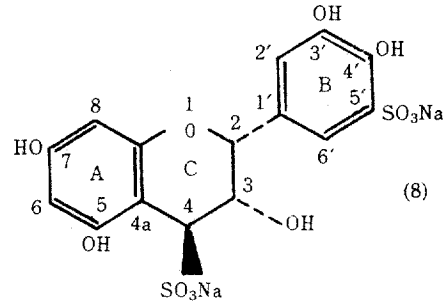
The major fraction eluted from this column was still a mixture as indicated by TLC analyses. The sample was evaporated, redissolved and then reapplied to a Sephadex LH-20 column.

This time the eluting solvent was 95% ethanol-water(1 : 1 v/v). Two fractions were obtained by this column. The first fraction which showed a R_f of 0.82 on TLC was evaporated and freeze dried to give 24mg of disodium epicatechin-(4β, 5′)-disulfonate.

Found : R_f 0.82. FAB-MS gave $[M-Na]^-$ and $[M-H]^-$ peaks at m/z 471 and 493. IR(cm^{-1}) : 3260, 1627, 1384, 1196, 1160, 1041, 645. ^1H NMR(δ) : 4.16(^1H, s, for H-4), 4.54(^1H, s, for H-3), 5.46(^1H, s, for H-2), 6.02(^1H, d, J=2.0Hz, for H-6), 6.05(^1H, d, J=2.7Hz, for H-8), 7.07(^1H, d, J=1.8Hz, for H-6′), 7.39(^1H, d, J=1.8Hz, for H-2′), ^{13}C NMR(ppm) : 61.35(C-4), 67.29(C-3), 76.12(C-2), 97.20(C-8), 97.75(C-6), 99.37(C-4a), 117.29(C-2′), 117.40(C-6′), 129.52(C-5′), 131.14(C-1′), 142.85(C-4′), 146.60(C-3′), 157.87, 159.21, 159.71(C-5, C-7 and C-8a).

The residual fractions were collected, freeze dried and stocked for further analyses.

3. RESULTS AND DISCUSSION



In the present work, disodium epicatechin-(4β, 5′)-disulfonate, a new compound, was isolated from the reaction mixture of sulfonates.

This reaction mixture derived from Douglas-fir condensed tannins was applied to a column of Sephadex LH-20 and three different solvents were sequentially used to separate this compound : 95% ethanol→aqueous ethanol(7 : 3 v/v)→aqueous ethanol(1 : 1 v/v).

A single spot on TLC was light pink (vanillin-HCl) and the R_f value was much higher(0.82) than that of epicatechin sulfonate(0.77) because of increased of polarity and water solubility due to the two inoic sodium sulfonate moieties.

An IR band at 1160cm^{-1} is attributed to the sulfonate groups(11, 16). In the negative ion FAB-MS spectrum, signals for $[M-Na]^-$ at m/z 471 and $[M-H]^-$ at m/z 493(Figure 2) were observed.

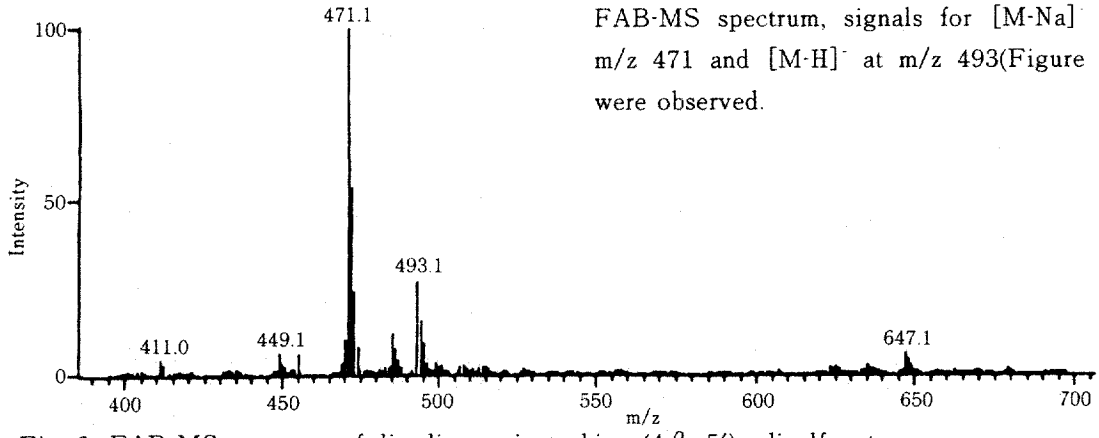


Fig. 2. FAB-MS spectrum of disodium epicatechin-(4β, 5′)-disulfonate.

The ^{1}H NMR spectrum(Figure 3) showed two doublets at δ 7.07(H-6′, J=1.87Hz) and δ 7.39(H-2′, J=1.85Hz) in the region of the catechol B ring protons.

These two protons are meta to each other as indicated by the small coupling constant(< 2Hz).

The H-2′ is lower field than the H-6′ due to the shielding effect on the H-6′ by the sodium sulfite substituent attached to C-5′.

The B ring proton signals are shifted downfield by 0.24ppm(H-6′) and 0.41ppm(H-2′) compared with the protons of epicatechin sulfonate owing to the electronegativity of the 5′-sodium sulfite moiety.

Signals for the two A ring protons were observed as two doublets at δ 6.02(H-6, J= 2.15Hz) and δ 6.05(H-8, J=2.70Hz).

The small coupling constants again showed two protons to be meta-coupled each other. In the heterocyclic C ring, the H-2, H-3 and H-4 signals were observed at δ 5.46, δ 4.54 and δ 4.16, respectively.

These proton signals are very similar to the assignments made by McGraw et al(8). The ^{13}C NMR spectrum is shown in Figure 4.

The C-4(61.35ppm) holding the sodium sulfite group showed a signal shifted downfield compared with the C-4 of epicatechin(28.6 ppm).

The C-2 and C-3 signals were observed at 76.12 and 67.29ppm.

The C-2 was also shifted upfield by 2.26ppm due to the γ-gauche effect from the C-4 sodium sulfite group(2, 10, 14).

The C-6, C-8 and C-4a of A ring showed

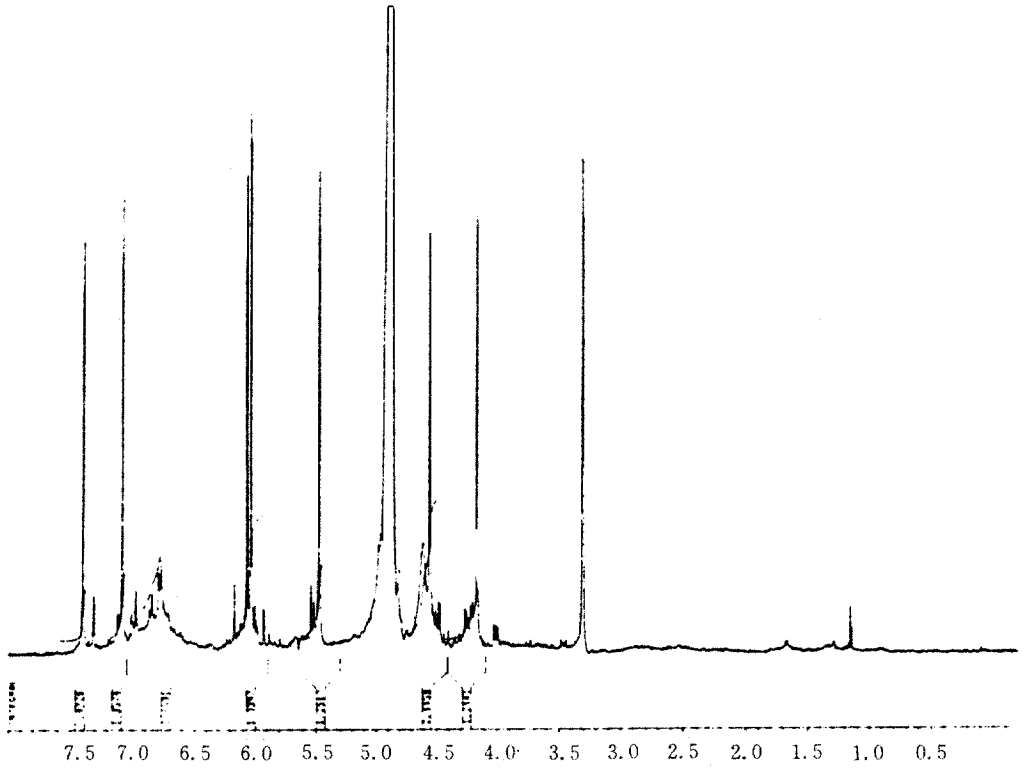


Fig. 3. ^{1}H NMR spectrum of disodium epicatechin−(4β, 5′)−disulfonate.

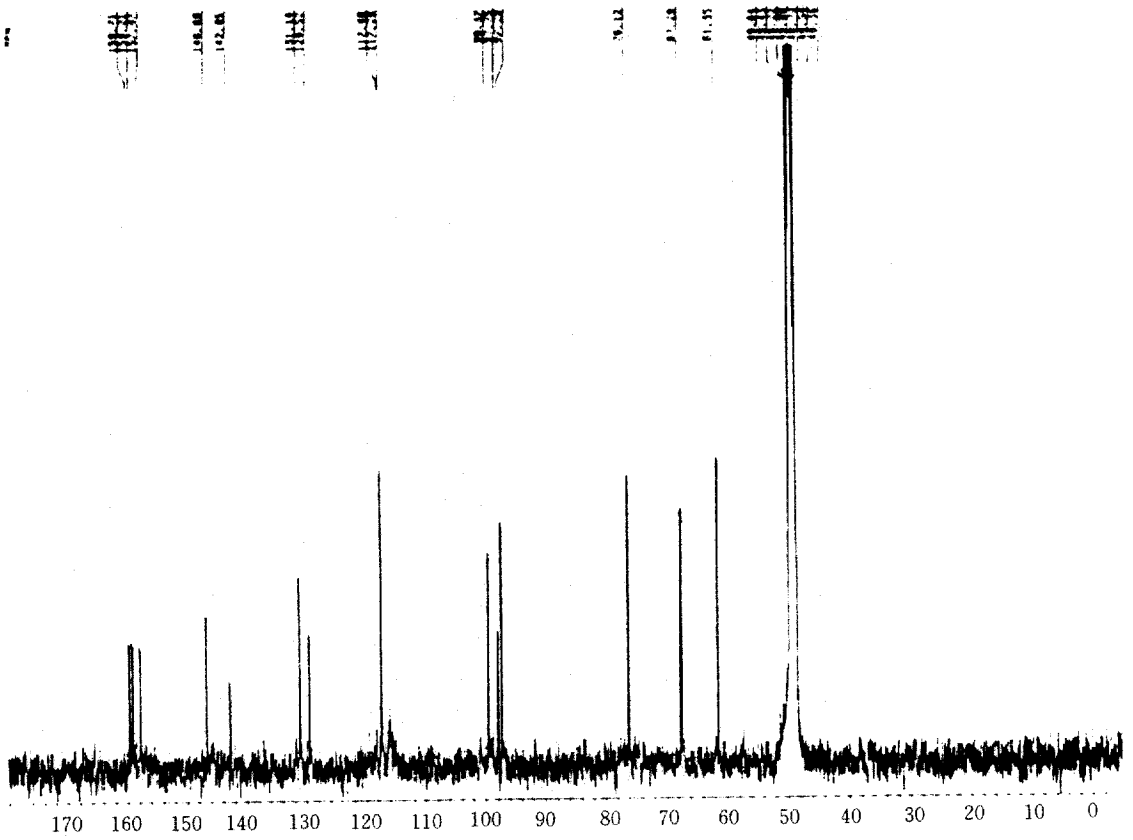


Fig. 4. ^{13}C NMR spectrum of disodium epicatechin−(4β, 5′)−disulfonate.

signals at 97.75, 97.20 and 99.37ppm, respectively.

The three quarternary carbons linked with oxygen in the A ring were in the region 157.87-159.71ppm. The chemical environment of the catechol B ring was very much changed because of the substitution with the sodium sulfite molecule at the C-5′.

The C-5′ at 115.95ppm is shifted downfield by 13.57ppm due to its bonding with the sulfite group.

Signals for C-3′ and C-4′, the quarternary carbons attached to oxygen, were observed at 146.60 and 142.85ppm.

The C-3′ signal was shifted the lower field than the C-4′. This may be attributed to the steric effect(7) or the shielding effect(13) on the C-4′ of the adjacent sulfite substituent.

The C-3′ signal was shifted also downfield by at least 0.64ppm compare with C-3′ in an unsubstituted B ring and this fact means that the electronegativity effect from the sodium sulfite group on the C-3′ may be greater than the steric or the shielding effect.

The C-2′ and C-6′ signals were observed at 117.29 and 117.40ppm and could not be distinguished from one another.

But the C-2′ signal was shifted downfield by at least 1.89ppm and the C-6′ signal was shifted upfield by at least 2.04ppm compare with those of an unsubstituted B ring.

This facts may be due to the paramagnetic effect(4) of the sulfite substituent. The C-1′ of the B ring showed a signal at 131.14ppm.

Consequently, the compound was identified as sodium epicatechin-(4β, 5′)-disulfonate.

The formation of this compound is likely to proceed through both radical reaction of the 4′ hydroxyl group of B ring and the formation of quinone methide intermediate of A ring.

Kennedy et al(6) have suggested that the epimerization of catechin or epicatechin at C-2, and the formation of catechinic acid in basic solution, may proceed through a radical rather than ionic mechanism on contrast to earlier results and Watanabe et al(15) also have reported that in radical sulfonation of lignin, the reaction was conducted basically by a combination of a sulfite ion radical and a lignin radical formed by the reaction of an oxiding agent-sulfite ion-lignin system. The proposed reaction scheme is shown in Figure 5.

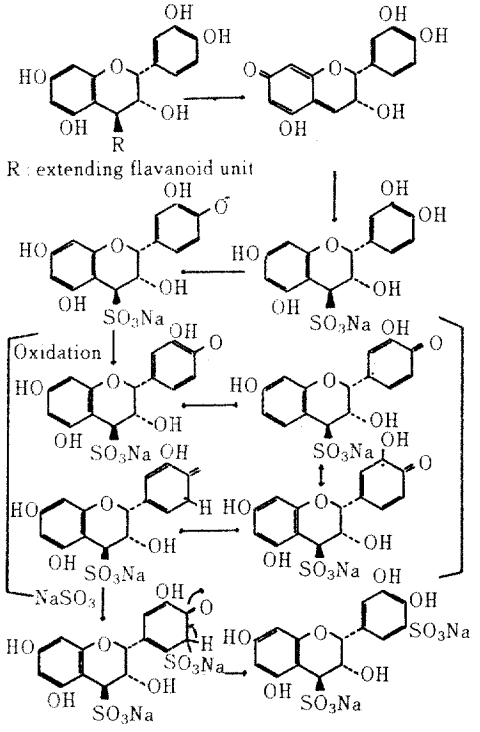


Fig. 5. The proposed reaction mechanism for the formation of disodium epicatechin −(4β, 5′)−disulfonate.

4. CONCLUSION

One reaction product from the sulfonation reaction was isolated and structurally elucidated by use of ^{1}H and ^{13}C NMR, IR and FAB-Mass spectrometry.

Isolation and purification involved the use of repeated column chromatography over Sephadex LH-20 with ethanol and various ethanol-water(7 : 3, 1 : 1 v/v) compositions as the eluting solvents.

The compound identified was disodium epicatechin-(4β, 5′)-disulfonate, new and novel structure that have not previously been reported.

This compound is the first example of electrophilic substitution of procyanidins under such mild sulfonation conditions.

A mechanism which involves the formation of a quinone methide intermediate of the catechol B ring is proposed.

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