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Degradation of (+)-Cyanidanol-3 by Sodium Sulfite in Aqueous Solution. II. Reactivity of Several (+)-Cyanidanol-3 Derivatives with Sodium Sulfite[1)]

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The reactivity of several (+)-cyanidanol-3 (cianidanol) derivatives with sodium sulfite in aqueous solution was investigated at pH 7.5 and 60 °C. 5,7-*O*-Dimethylcianidanol was degraded by sodium sulfite to yield a water-soluble degradation product, which was assumed to be 5,7-*O*-dimethylepicatechin carrying the sodium sulfonate function in place of the aliphatic hydroxy group at the C-3 position. The degradation by sodium sulfite was inhibited by the addition of boric acid and by lowering the pH of the solution to 3.0. On the other hand, 3′,4′-*O*-dimethylcianidanol and 5,7,3′,4′-*O*-tetramethylcianidanol were very stable in aqueous solution containing sodium sulfite. The mechanism of the attack of sulfite ion and/or bisulfite ion at the C-3 position of the dissociated form of cianidanol or 5,7-*O*-dimethylcianidanol is discussed.

Keywords——(+)-cyanidanol-3; (+)-5,7-*O*-dimethylcianidanol; (+)-3′,4′-*O*-dimethylcianidanol; (+)-5,7,3′,4′-*O*-tetramethylcianidanol; stability; degradation; hepatoprotector; sulfite

Since the inactivation of epinephrine by sodium bisulfite was reported by Higuchi *et al.*,[2)] several investigations on the reactivity of medicaments with antioxidant have been presented. However, most of the reports dealt primarily with the lowering of medicament content in aqueous solution containing an antioxidant. So far, only the degradation products of epinephrine,[2a)] physostigmine,[3)] dexamethasone-21-phosphate,[4)] morphine[5)] and so on by sodium bisulfite, which has been widely used as an antioxidant, have been investigated in detail. In many cases, a medicament which has a catechol moiety in its structure undergoes auto-oxidation, and an antioxidant such as sodium sulfite or sodium bisulfite has therefore been required in preparing such a medicament as solutions. However, little work has been done on the reactivity of medicaments containing a catechol moiety with antioxidant, except for the study reported by Higuchi *et al.*,[2b)] who investigated the reactivity of some medicaments containing a phenolic group or catechol group with sodium bisulfite.

We showed in a previous report[6)] that (+)-cyanidanol-3 (cianidanol) is decomposed by sodium sulfite to afford a water-soluble degradation product which was assumed to be cianidanol carrying the sodium sulfonate function in place of the aliphatic hydroxy group at the C-3 position. In this study, we investigated the reactivity of several cianidanol derivatives with sodium sulfite to clarify the mechanism of the degradation of cianidanol by sodium sulfite.

Experimental

Materials——Cianidanol (**1**) was obtained from Zyma S. A. and recrystallized from water, then dried over phosphorus pentoxide. (±)-Epicatechin was obtained from Zyma S. A. as a standard sample and used as supplied.

5,7-*O*-Dimethylcianidanol (**2**) was synthesized according to the procedure reported by Hathway *et al.*,[8)] white needles (from aqueous ethanol), mp 211—213 °C (dec.) (reported 218—219 °C[8)]).

3′,4′-*O*-Dimethylcianidanol (**3**) was synthesized *via* the catalytic reduction of 5,7-*O*-dibenzyloxycarbonyl-3′,4′-*O*-dimethylcianidanol. 5,7-*O*-Dibenzyloxycarbonylcianidanol was prepared according to the procedure described in the Japanese patent.[7] Ten grams of 5,7-*O*-dibenzyloxycarbonylcianidanol was dissolved in 80 ml of ethyl acetate, and then an ether solution of diazomethane was added in large excess. After being stirred at room temperature for 1 h, the reaction mixture was evaporated under reduced pressure and the residue was dissolved in 200 ml of ethyl acetate. The catalytic reduction of this solution was carried out in the presence of 1 g of palladium carbon and hydrogen at room temperature and 4 atmospheres pressure. The reaction mixture was filtered to remove palladium carbon and the filtrate was evaporated under reduced pressure to afford a yellow syrup. The purification of this crude product by silica gel column chromatography and recrystallization from chloroform–methanol yielded white crystals (3.9 g), mp 246—247 °C (dec.). Nulcear magnetic resonance (NMR) (DMSO-d_6) δ: 2.32—2.72 (2H, m, 4-CH_2), 3.70 (6H, s, -OCH_3), 3.92 (1H, m, 3-CH), 4.59 (1H, d, J=9 Hz, 2-CH), 4.77 (1H, m, 3-OH), 5.62 (1H, d, J=2 Hz, 6-arom.), 5.82 (1H, d, J=2 Hz, 8-arom.), 6.72 (3H, br s, 2′,5′,6′-arom.), 8.22 and 8.96 (2H, two s, phenolic OH).

5,7,3′,4′-*O*-Tetramethylcianidanol (**4**) was synthesized according to the procedure reported by Mehta *et al.*,[9] white needles, mp 141—142 °C (dec.) (reported 142—143 °C[9]). NMR ($CDCl_3$) δ: 1.78 (1H, d, J=4 Hz, 3-OH), 2.30—3.35 (2H, m, 4-CH_2), 3.77, 3.82, 3.89 (12H, three s, -OCH_3), near 4.1 (1H, m, 3-CH), 4.68 (1H, d, J=9 Hz, 2-CH), 6.15 (2H, s, 6,8-arom.), 6.97, 6.99 (3H, two s, 2′,5′,6′-arom.).

(±)-5,7-*O*-Dimethylepicatechin was prepared by *O*-methylation of (±)-epicatechin according to a procedure similar to that used in the preparation of 5,7-*O*-dimethyl cianidanol. (±)-Epicatechin (1.5 g) was dissolved in 100 ml of water and the pH of the solution was adjusted to 12.3 with 1 N NaOH. Then 4 ml of dimethyl sulfate was added to the solution and the whole was shaken for 10 min at room temperature. The reaction mixture was cooled to 0—5 °C in an ice bath, and the pH was adjusted to 3.1 with 1 N HCl. The reaction mixture was extracted with three 100 ml portions of ethyl acetate. The extracted solution was washed with water, dried over $MgSO_4$ and concentrated under reduced pressure to afford a yellow syrup (0.9 g). Further purification by preparative thin-layer chromatography (TLC) afforded a yellow solid (0.45 g) [Kieselgel 60 F_{254} developed with chloroform–methanol (85:15)]. Recrystallization from chloroform–methanol failed to yield crystals. mp 146—149 °C (dec.). NMR (DMSO-d_6) δ: 2.4—2.8 (2H, m, 4-CH_2), 3.73—3.77 (6H, two s, -OCH_3), 3.90—4.25 (1H, m, 3-OH), 4.80 (1H, br s, 2-CH), 6.0—6.4 (2H, m, 6,8-arom.), 6.74 (2H, s, 5′,6′-arom.), 6.98 (1H, s, 2′-arom.), 8.78 (3H, br s, phenolic OH).

Other chemicals were of reagent grade quality. Deionized and distilled water was used in all experiments.

Stability Studies——a) Degradation of Cianidanol Derivatives in Aqueous Solution: A cianidanol derivative, **2**, **3**, or **4**, was dissolved in phosphate buffer solution, pH 7.0, at a concentration of 0.2 w/v%, in the presence of sodium sulfite (0.032 or 0.16 M). Dioxane was used at concentrations of 20 and 30 v/v% as an aid to dissolve **2** and **4**, respectively. On the other hand, dimethyl sulfoxide was used to dissolve **3** at a concentration of 40 v/v%. The pH of each buffer solution was adjusted to 7.5 with conc. HCl. The prepared sample solutions were then heated in a temperature controlled water bath at 60±0.2 °C.

b) Effect of Boric Acid on the Degradtion of **2** by Sodium Sulfite: **2** was dissolved in a phosphate buffer of pH 8.5 at a concentration of 6.2×10^{-3} M, in the presence of sodium sulfite (0.16 M) and boric acid (0.021 M). In this case also, dioxane had been added at 20 v/v% to dissolve **2**. The pH of the sample solution was adjusted to 8.5 with conc. HCl and the solution was heated at 60±0.2 °C.

c) Effect of pH on the Degradation of **2** by Sodium Sulfite: **2** was dissolved in a glycine buffer of pH 3.0 or phosphate buffer of pH 7.5 at a concentration of 6.2×10^{-3} M, in the presence of sodium sulfite (0.16 M). Dioxane was used at 20 v/v% as an aid to dissolve **2**. The pH values of the sample solutions were adjusted to 3.0 and 7.5,

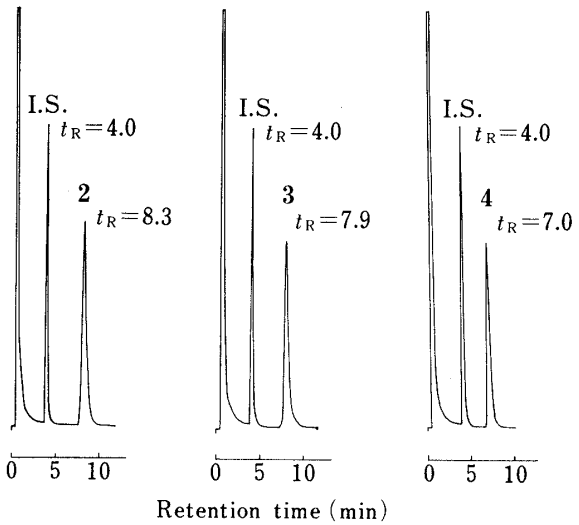


Fig. 1. Gas Chromatograms of Trimethylsilylated **2**, **3**, **4** and Internal Standard (I.S.)

A 2% silicone UC W-98 2 m glass column was used: column temp. 250 °C; injection port and detector temp. 310 °C.

respectively, with conc. HCl. The prepared sample solutions were then heated at 60±0.2 °C.

Assay Method——A 5 ml aliquot of the degraded solution was mixed with 10 ml of ethyl acetate. In the case of the solution containing boric acid, 1 ml of 1 N HCl was added before the ethyl acetate. The whole was shaken and centrifuged. Two milliliters of the organic layer was evaporated to dryness, and the residue was dissolved in 1 ml of internal standard solution containing 1 ml of pyridine and 1 mg of dehydroepiandrosterone. To this solution, 250 μl of bis(trimethylsilyl)-trifluoroacetamide was added. The reaction mixture was allowed to stand for 30 min, and then 1 μl was injected into a gas-liquid chromatograph. Gas-liquid chromatography was performed on a 2 m × 3 mm i.d. glass column packed with 2% silicone UC W-98 on Chromosorb W (AW-DMCS). The injection port, column and detector temperatures were maintained at 310, 250 and 310 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 50 ml/min. Preliminary experiments revealed that intact cianidanol derivatives and their epimers were well separated under these conditions. Satisfactorily separated peaks of trimethylsilylated **2**, **3** and **4**, and internal standard, were obtained (Fig. 1). Quantitation was based on peak height ratio comparison with linear standard plots.

Isolation of the Degradation Product Generated by Addition of Sodium Sulfite to the Aqueous Solution of 2——Two grams of 5,7-*O*-dimethylcianidanol was dissolved in 200 ml of dioxane, then 800 ml of sodium sulfite aqueous solution (2.6×10^{-1} M) was added, and the pH of the whole was adjusted to 7.5 with conc. HCl. The solution was heated at 60 °C for 66 h and the residue obtained by evaporation was shaken with 200 ml of acetone. The mother liquor obtained by filtration was dried over $MgSO_4$, and then evaporated under reduced pressure to give a white solid (1.1 g). Recrystallization from acetone–ether afforded white needles, yield 0.7 g, mp 215—220 °C (dec.).

Results and Discussion

Stability of Cianidanol Derivatives in Aqueous Solution Containing Sodium Sulfite

The stability of cianidanol derivatives, **2**, **3** and **4**, in aqueous solution containing sodium sulfite was examined at pH 7.5 and 60 °C. As shown in Fig. 2, the sum of the concentrations of **2** and its epimer in the aqueous solution containing sodium sulfite decreased with time, compared with that in the control solution which was free from sodium sulfite. Further, **2** was

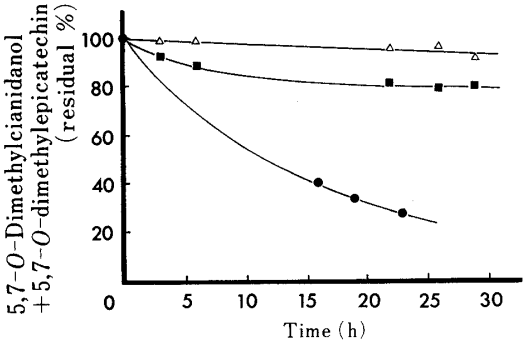


Fig. 2. Reactivity of 5,7-*O*-Dimethylcianidanol (**2**) (6.3×10^{-3} M) with Sodium Sulfite in Aqueous Solution Containing Dioxane (20 v/v%) at pH 7.5 and 60 °C

—△—, without Na_2SO_3; —■—, Na_2SO_3 3.2×10^{-2} M; —●—, Na_2SO_3 1.6×10^{-1} M.

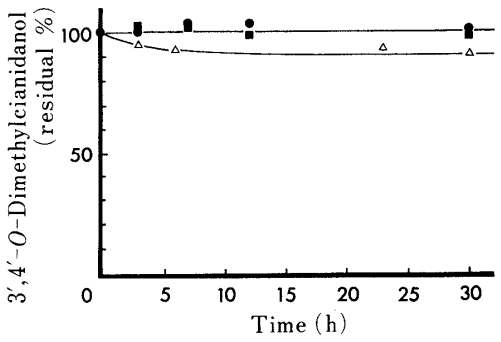


Fig. 3. Reactivity of 3′,4′-*O*-Dimethylcianidanol (**3**) (6.3×10^{-3} M) with Sodium Sulfite in Aqueous Solution Containing Dimethyl Sulfoxide (40 v/v%) at pH 7.5 and 60 °C

—△—, without Na_2SO_3; —■—, Na_2SO_3 3.2×10^{-2} M; —●—, Na_2SO_3 1.6×10^{-1} M.

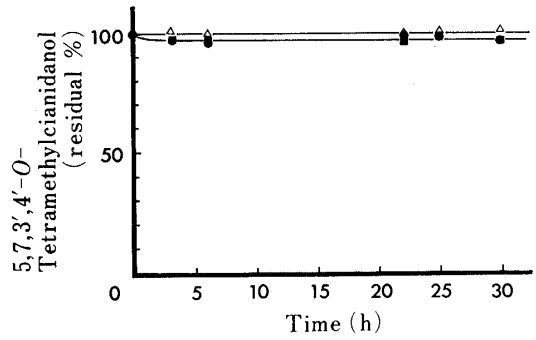


Fig. 4. Reactivity of 5,7,3′,4′-*O*-Tetramethylcianidanol (**4**) (5.8×10^{-3} M) with Sodium Sulfite in Aqueous Solution Containing Dioxane (30 v/v%) at pH 7.5 and 60 °C

—△—, without Na_2SO_3; —■—, Na_2SO_3 3.2×10^{-2} M; —●—, Na_2SO_3 1.6×10^{-1} M.

more labile with increasing concentration of sodium sulfite. The overall concentration change of **2** and its epimer is plotted in Fig. 2 since **2** was found to be converted to its epimer even in the control solution free from sodium sulfite at this pH, as discussed below. On the other hand, **3** was more stable in the aqueous solution containing sodium sulfite than in the control solution because of the antioxidant action of sodium sulfite (Fig. 3). Compound **4** was very stable in both the aqueous solution containing sodium sulfite and the control solution free from sodium sulfite, as shown in Fig. 4.

Identification of the Degradation Product Generated by Addition of Sodium Sulfite

The degraded solution (initial concentrations of **2** and sodium sulfite were 6.6×10^{-3} and 1.6×10^{-1} M, respectively), after being heated at 60 °C and pH 7.5 for 15 h, was subjected to TLC on a cellulose plate (Cellulose F, Merck) using H_2O–dioxane–acetic acid (10 : 1 : 1) as the developing solvent. The products were detected by means of 10% Na_2CO_3 aq. and 0.2% Echtblausalz B (Merck) aq. spray. Two spots of products, other than **2** (*Rf* 0.64), were detected at *Rf* 0.55 and 0.84. The *Rf* value of one of the degradation products (*Rf* 0.55) was identical with that of 5,7-*O*-dimethylepicatechin, which was prepared independently from (±)-epicatechin according to the procedure described in the experimental section. Further, the retention time of this degradation product on gas-liquid chromatography (see Experimental) was identical with that of 5,7-*O*-dimethylepicatechin similarly treated (retention time 7.6 min). However, further isolation and identification by other analytical methods could not be carried out because of the low yield. On the other hand, the degradation product of *Rf* 0.84, which was not generated in the control solution free from sodium sulfite, was isolated according to the procedure described in the experimental section. Quantitative analysis of the isolated degradation product was carried out as follows. After carbonization of the isolated degradation product, water was added to the residue, and the whole was stirred and filtered. An aqueous solution of barium chloride was added to the mother liquor to produce a white precipitate. This precipitate was insoluble in dilute nitric acid. In addition, the presence of sodium salt was revealed by the flame reaction. These results suggested that a sodium sulfonate function was present in the degradation product, as in the degradation product generated from cianidanol by sodium sulfite reported in the previous work.[6] Since the signal of the aliphatic hydroxy group of **2** at the C-3 position was not detected in the NMR spectrum

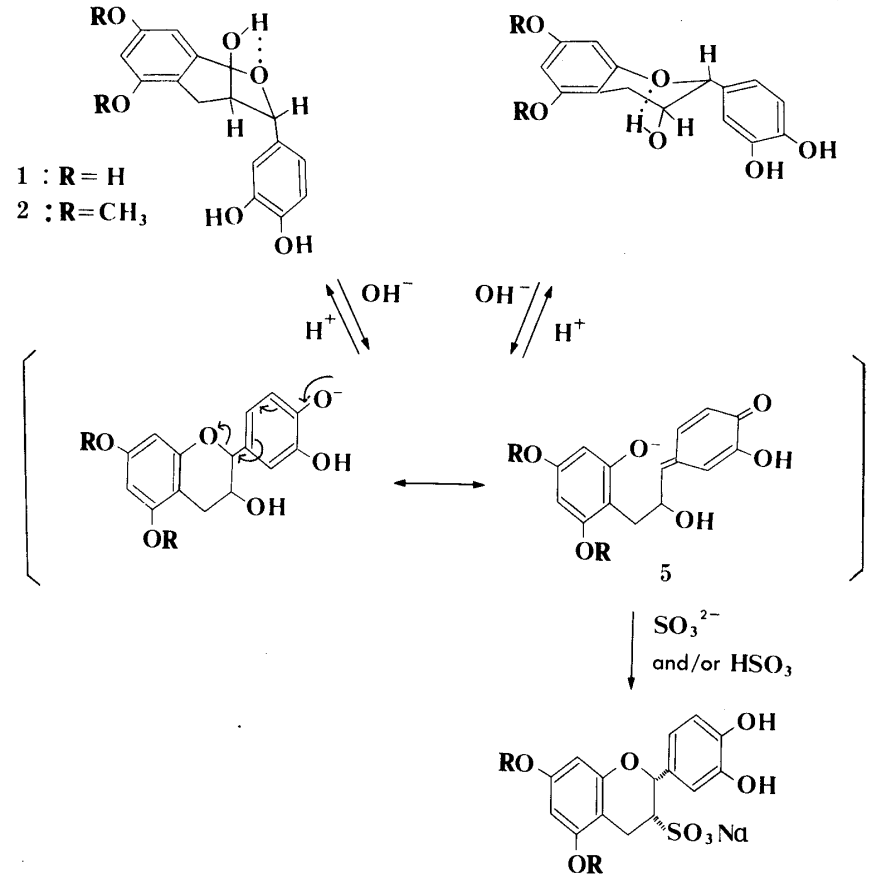
Table I. NMR Spectral Data for 5,7-*O*-Dimethylcianidanol and the Degradation Product by Na_2SO_3 (δ Values, ppm)[a]

	4-CH_2	5,7-OCH_3	3-CH	2-CH	3-OH
5,7-*O*-Dimethyl-cianidanol	2.24—2.84 (2H, m)	3.68, 3.73 (6H, two s)	3.65—4.01 (1H, m)	4.55 (1H, d, J=8 Hz)	4.91[b] (1H, d, J=2 Hz)
Degradation product by Na_2SO_3	2.32—2.60 (2H, m)	3.56, 3.68 (6H, two s)	4.16—4.40 (1H, m)	3.44 (1H, d, J=8 Hz)	n.d.[c]

	6-arom.	8-arom.	5′,6′-arom.	2′-arom.	Phenolic OH
5,7-*O*-Dimethyl-cianidanol	6.03 (1H, d, J=2 Hz)	6.12 (1H, d, J=2 Hz)	6.62—6.70 (2H, m)	6.72—6.78 (1H, m)	8.78[b] (2H, br s)
Degradation product by Na_2SO_3	5.94—6.08 (2H, m)		6.58—6.70 (2H, m)	6.80—6.88 (1H, m)	8.63[b] (2H, br s)

a) Measured at 100 MHz with TMS as an internal standard.
b) Not detected after addition of D_2O.
c) Not detected.

Chart 1



of the degradation product (Table I), the degradation product was assumed to be **2** containing the sodium sulfonate function in place of the aliphatic hydroxy group at the C-3 position. There was a downfield shifts of the 2′-aromatic proton in the NMR spectrum of the degradation product in comparison with the signal of **2**. The signal of the 2′-aromatic proton of (−)-epicatechin was reported to show a marked downfield shift compared with that of cianidanol.[10] Consequently, the stereochemistry of the degradation product at the C-2 and C-3 position was assumed to be *cis*, as shown in Chart 1, by analogy with the degradation product generated from cianidanol (**1**) by sodium sulfite.

Effect of pH on the Degradation of 2 by Sodium Sulfite

To investigate the effect of pH on the degradation of **2** by sodium sulfite, **2** (6.2×10^{-3} M) and sodium sulfite (0.16 M) were dissolved in buffer solutions of pH 7.5 and 3.0, and the solutions were heated at 60 °C. As shown in Fig. 5, the sum of the concentrations of 5,7-*O*-dimethylepicatechin and **2** in the aqueous solution containing sodium sulfite decreased markedly with time at pH 7.5, compared with that in the control solution. In contrast, no degradation of **2**, including epimerization, was observed even in the aqueous solution containing sodium sulfite. In the preliminary work, it was found that cianidanol was little degraded by sodium sulfite at pH 3.0, where the epimerization to epicatechin was not observed in aqueous solution free from sodium sulfite. These results suggested that the degradation of cianidanol and **2** by sodium sulfite occurs at pH values where epimerizations of cianidanol and **2** occur easily.

Effect of Boric Acid on the Degradation of 2 by Sodium Sulfite

Boric acid forms a protecting group for the catechol moiety. To examine the effect of

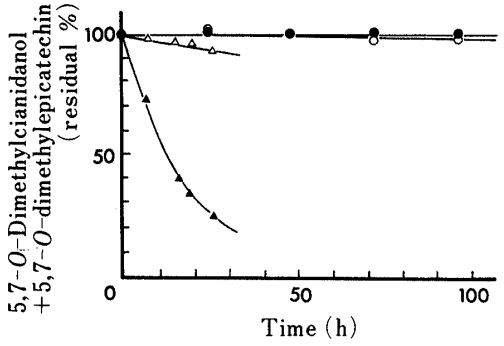


Fig. 5. Effect of pH on the Degradation of 5,7-*O*-Dimethylcianidanol (**2**) by Sodium Sulfite in Aqueous Solution Containing Dioxane (20 v/v%) at 60 °C

—○—, without Na_2SO_3 at pH 3.0; —●—, Na_2SO_3 1.6×10^{-1} M at pH 3.0; —△—, without Na_2SO_3 at pH 7.5; —▲—, Na_2SO_3 1.6×10^{-1} M at pH 7.5.

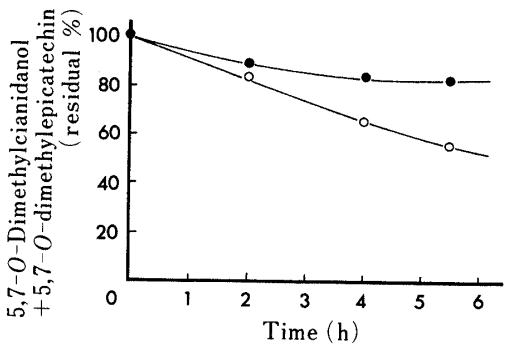


Fig. 6. Effect of Boric Acid on the Degradation of 5,7-*O*-Dimethylcianidanol (**2**) (6.3×10^{-3} M) by Sodium Sulfite (0.16 M) in Aqueous Solution Containing Dioxane (20 v/v%) at pH 8.5 and 60 °C

—○—, without H_3BO_3; —●—, H_3BO_3 2.1×10^{-2} M.

boric acid on the degradation of **2** by sodium sulfite, boric acid (2.1×10^{-2} M) was added to the aqueous solution of **2** (6.2×10^{-3} M) containing sodium sulfite (0.16 M) and the whole was heated at 60 °C and pH 8.5. As shown in Fig. 6, the degradation of **2** by sodium sulfite was markedly inhibited by the addition of boric acid. With respect to the degradation of cianidanol by sodium sulfite, a similar effect of boric acid was observed at pH 8.0 in preliminary investigations. Formation of cyclic borate (or *O*-methylation in the cases of **3** and **4**) protects the 3′,4′-phenolic hydroxy groups, and this appears to inhibit attack of HSO_3^- or SO_3^{2-} on the carbon atom at the C-3 position.

Conclusion

Compound **2** was degraded by sodium sulfite in neutral or basic aqueous solution to yield a water-soluble degradation product; this was concluded to be 5,7-*O*-dimethylepicatechin in which a sodium sulfonate moiety replaces the aliphatic hydroxy group at the C-3 position. Further, the degradation of **2** was inhibited by the addition of boric acid and by lowering the pH of the solution to the acidic region. On the other hand, **3** and **4**, both of which are *O*-methylated in the catechol moiety, were very stable in aqueous solution containing sodium sulfite. From these results, the degradation of **1** and **2** was assumed to occur *via* the dissociated forms (**5**) as shown in Chart 1, since the steric hindrance around the C-3 carbon of both **1** and **2** is expected to be very large compared with that of the dissociated forms.[11] In addition, direct attack of HSO_3^- or SO_3^{2-} on this carbon atom of epicatechin or 5,7-*O*-dimethylepicatechin is likely to be difficult, because epicatechin was not degraded by sodium sulfite at pH 3, where epimerization of epicatechin to **1** did not occur. Consequently, it is suggested that the attack of sulfite ion or bisulfite ion on the carbon atom at the C-3 position of **1** or **2** only occurs on the dissociated form of **1** or **2**.

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References and Notes

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