Studies on the Constituents of Aloe arborescens Mill. var. natalensis BERGER. II. The Structures of Two New Aloesin Esters

KENJI MAKINO, AKIRA YAGI, and ITSUO NISHIOKA

Faculty of Pharmaceutical Sciences, Kyushu University

(Received December 14, 1973)

Two new aloesin esters were isolated from the fresh leaves of Aloe arborescens Mill. var. natalensis BERGER, and their structures were established to be 2"-O-p-coumaroylaloesin and 2"-O-feruloylaloesin on the basis of chemical and spectral evidences. These esters are the first naturally occurring 2"-O-acylated C-glucosyl compounds.

In the previous paper we reported the structure of aloearbonaside which was a novel chromenylglucoside in Aloe arborescens Mill. var. natalensis BERGER. Recently, Haynes, et al., isolated a new C-glycosyl derivative, 2-acetonyl-8-glucopyranosyl-7-hydroxychromone, named aloesin, from Aloe spp. Independently, Wagner, et al., reported the isolation of two C-glucosylchromones, aloesin A ("Aloe Harz") and aloesin B, the latter of which had the same structure to aloesin, and presumed aloesin A to be 6"-O-p-coumaroylaloesin. This paper deals with the structure determination of two new aloesin esters; 2"-O-p-coumaroylaloesin (I) and 2"-O-feruloylaloesin (II), isolated from Aloe arborescens Mill. var. natalensis BERGER. These are the first 2"-O-acylated C-glycosyl compounds to be described.

The concentrated MeOH extract was treated with acetone and the acetone insoluble portion was worked up by repeated silica gel column chromatography using acetone–EtOAc and CHCl₃–EtOH as solvents to give compound B (II). The fraction eluted with acetone–EtOAc

![Chart 1](image-url)
was rechromatographed over a silica gel column, followed by preparative thin-layer chromatography (TLC) and recrystallization to give compound A (I).

I, mp 156—162°, C_{28}H_{38}O_{11}, [\alpha]_{D}^{20} = 82.7°, showing blue fluorescence under ultraviolet (UV) lamp, indicated the positive coloration reaction to Echtblausalz B-KOH and sodium nitroprusside reagent. The UV spectrum (288, 244, 253 and 300 nm) and the infrared (IR) spectrum (3400, 1715 and 1650 cm⁻¹) of I revealed a remarkable similarity to those of aloeosin.⁷

The nuclear magnetic resonance (NMR) spectrum of I exhibited two vinyl proton signals (J=16 Hz) at δ 6.10 and 7.35, two pairs of AB splitting aromatic proton signals (J=8 Hz) at δ 6.75 and 7.45, and phenolic proton signals at δ 9.95 and 10.68, besides the proton signals due to aloeosin. The presence of ester bond in I was suggested by IR spectral analysis (1715 cm⁻¹) and by the positive coloration to hydroxamate–ferric chloride. On hydrolysis with 10% HCl in dioxane–H₂O (1:1) I gave aloeosin (III), mp 142—144° and p-coumaric acid which were identified by the direct comparison (UV, IR, NMR, TLC and mixed melting point) with an authentic sample, respectively. Accordingly, I was confirmed to be mono p-coumaroyl-aloeosin.

The location of p-coumaroyl moiety in I was determined in the following way. Since the phenolic proton signals appeared at δ 9.95 and 10.68 in NMR spectrum of I, it was suggested that the acyl residue was linked at the hydroxyl group in sugar moiety.

When tritylated with trityl chloride in pyridine I gave trityl ether (IV), mp 140—144°, C_{42}H_{38}O_{11}·1\text{/}_3H₂O indicative of the characteristic trityl group at δ 7.2—7.6 in NMR spectrum and at 700 cm⁻¹ in IR spectrum, and it was verified that the acyl group did not connect with a primary alcohol.

On treatment with benzaldehyde and anhydrous ZnCl₂ I provided benzylidene derivative (V), mp 218—222°, C_{32}H_{32}O_{11}·2H₂O exhibiting the aromatic proton signals at δ 7.2—7.6 and a benzylidene methine proton signal at δ 6.64 in the NMR spectrum. These observations excluded the possibility for the location of the acyl group at C₄‴ or C₄‴ in sugar moiety.

---

⁷ The β-linkage of sugar in aloeosin has been demonstrated by doublets (J=9 Hz) due to the anomeric proton at δ 4.689 and the spin decoupling experiment of hexacetate (IIia).⁸


⁹ By the spin decoupling experiments the methine and methylene proton signals in IIa were assigned as follows: δ 4.20 (C₄‴-H, m.), 5.20 (C₄‴-H, m.), 5.82, 5.88 (C₄‴ or C₄‴-H, t, J=8 Hz) and 5.62 (C₄‴-H, d, J=8 Hz).

Oxidation of I with periodate at 5° resulted in the consumption of 1.05 mole equivalent of periodate during 24 hr. Therefore, the above findings led to the conclusion that \( p \)-coumaric acid must be attached at \( C_4'' \) of sugar moiety in I. This was in good accord with the following NMR spectral examination on the acetate (Ia).

The NMR spectrum of hexaacetylasoerin (IIa), mp 116—118°, indicated acetyl proton signals at \( \delta 1.72 \) (C\( _2'' \)), 2.04 (C\( _4'' \)), 2.08 (C\( _3'' \)), 2.28 (C-2') and 2.42 (C-7),\(^9\) while hexaacetate of I, (Ia), mp 124—138°, C\( _34 \)H\( _40 \)O\( _{17} \) exhibited acetyl proton signals at \( \delta 2.00 \) (C\( _4'' \)), 2.05 (C\( _3'' \)), 2.42 (C-7), 2.26 (C-4 in \( p \)-coumaroyl group) and 2.28 (C\( _8' \)).

Since the 2\(^{''}\)-O-acetyl methyl group in acetylated C-glicosyl-xanthone,\(^{11}\) flavone\(^{12}\) and -chromone\(^{4}\) can be expected to be affected by the magnetic anisotropy of the aromatic ring, the higher field signal (\( \delta 1.7—1.8 \)) than signals due to C\( _4'' \), C\( _3'' \), C\( _8' \)-OAc was assigned to the C\( _3'' \)-OAc.\(^{13}\) Indeed, the acetyl proton signal due to C\( _4'' \)-OAc appeared at \( \delta 1.72 \) in the NMR spectrum of IIa. However, in the NMR spectrum of Ia no diagnostic signal due to C\( _3'' \)-OAc could be detected. This information was consistent with the results of the above chemical evidences.

Consequently, the structure of compound A should be presented as 2\(^{''}\)-O-\( p \)-coumaroylaloesin (I).

II, mp 153—156°, C\( _{25} \)H\( _{39} \)O\( _{12} \)·H\( _2 \)O, \( [\alpha]_D^{25} \) —77.5° showing blue fluorescence under UV lamp, expressed the positive coloration reaction to Echtblausalz B·KOH, sodium nitroprusside and hydroxamate-ferric chloride, and presented almost identical absorption bands to those of I in UV spectrum (230, 244, 253, 300 and 320 nm) and IR spectrum (3400, 1715 and 1650 cm\(^{-1} \)). The NMR spectrum of II exhibited two phenolic proton signals at \( \delta 8.16 \) and 8.90, two vinyl proton signals (\( J=16 \) Hz) at \( \delta 6.06 \) and 7.30, a methoxyl proton signal at \( \delta 3.88 \) and ABX splitting three aromatic proton signals (\( J_{\text{orth}}=10 \) Hz, \( J_{\text{meta}}=2 \) Hz) at \( \delta 6.64 \), 6.94 and 7.18, besides the signals attributed to the protons of aloesin. Comparative studies on UV, IR and NMR spectra of I and II clearly revealed a close relationship between their structures.

On hydrolysis II yielded aloesin and ferulic acid which were identified with an authentic sample by the direct comparison (UV, IR, NMR, TLC and mixed melting point). Thus, the structure of II proved to be monoferuloylaesin. In the NMR spectrum of II the presence of two phenolic protons at \( \delta 8.16 \) and 8.90 revealed the hydroxyl group in sugar moiety was esterified.

The location of feruloyl moiety was determined by the similar manner to that of I.

The formation of trityl derivative (VI), mp 132—135°, C\( _{45} \)H\( _{44} \)O\( _{12} \)·2H\( _2 \)O indicative of the characteristic trityl group at \( \delta 7.1—7.5 \) (NMR) and at 700 cm\(^{-1} \) (IR), and of benzylidene derivative (VII), mp 188—191°, C\( _{36} \)H\( _{34} \)O\( _{12} \) revealing the aromatic proton signals at \( \delta 7.2—7.6 \) and a benzylidene methine proton signal at \( \delta 5.72 \) (NMR) led to the conclusion that ferulic acid was linkaged at C\( _2'' \) or C\( _3'' \) position.

Oxidation of II with periodate at 5° resulted in the consumption of 1.15 mole equivalent of periodate during 24 hr.

Thus, it was established that ferulic acid should be attached at C\( _2'' \) position in sugar moiety of II. The above chemical evidences agreed with the following NMR spectral examinations on the acetate (IIa).

The NMR spectrum of hexaacetate of II, (IIa), mp 106—108°, C\( _{44} \)H\( _{42} \)O\( _{18} \) indicated the acetyl proton signals at \( \delta 2.00 \) (C\( _2'' \)), 2.08 (C\( _3'' \), C\( _4'' \)), 2.24 (C\( _8' \)), 2.29 and 2.41 (two phenol ace-

tates). The absence of the diagnostic C_{\alpha''}-OAc proton signal led to the conclusion that feruloyl moiety must be attached at C_{\alpha''}-O position in sugar moiety.

Accordingly, the structure of compound B should be 2''-O-feruloylaloesin (II).

Although Haynes, et al., reported the presence of aloesin in Aloe spp. no aloesin was detected from the fresh leaves of this plant.

As the O-glycosides having \(p\)-coumaroyl or feruloyl functions at C-2 position in sugar moiety, grandidentatim and petunoside were reported. The C-glycosides with \(p\)-coumaroyl or feruloyl substituents at C-2 position in glucose moiety apparently are the first naturally occurring compounds.

Biogenetically, 2''-O-p-coumaroylaloesin and 2''-O-feruloylaloesin, together with barbaloin and aloearcabinoside were speculated to be derived from polyketides in this plant. The biosynthetic studies are in progress.

**Experimental**

Melting points were determined on a Yanagimoto melting point apparatus and uncorrected. IR spectra were obtained with a KOKEN DS-301 and UV spectra were recorded with a Shimadzu UV-50A. NMR spectra were taken with a JEOL C-60H or PS-100. Chemical shifts were expressed in ppm from Me, Si as internal reference and coupling constants (\(J\) in Hz). The spin decoupling experiments were demonstrated in CDC\(_6\) by JEOL PS-100. TLC were performed on silica gel (Merck) employing the following solvent systems: 1) EtOAc: MeOH: H\(_2\)O (20: 3: 2), 2) CHCl\(_3\): EtOH: H\(_2\)O (30: 7: 3), 3) CHCl\(_3\): EtOAc (2: 1). As a reagent 1% Echtblausalz B (Merck)–KOH solution and the blue fluorescence under UV light when alcoholic alkaline solution was sprayed, were used for detection.

**Isolation of 2''-O-p-Coumaroylaloesin (I) and 2''-O-Feruloylaloesin (II)** — The homogenate of the fresh leaves (5 kg), cultivated at the garden of this university, was extracted with MeOH (3 liter) at room temperature and the filtrate was concentrated to syrup. To the syrup acetone (5 liter) was added and the precipitate was removed by filtration. The filtrate was evaporated to dryness and the residue (14.5 g) was chromatographed over a silica gel column (150 g) using EtOAc and EtOAc–acetone (4: 1) subsequently as the solvent. The EtOAc–acetone (4: 1) eluate (Fraction A) (4.8 g) was chromographically over a silica gel column (100 g) using CHCl\(_3\) and CHCl\(_3\)–EtOH (9: 1) as the solvent. The CHCl\(_3\)–EtOH (9: 1) (2.1 g) eluate was chromographically over a silica gel column (70 g) using EtOAc and EtOAc–acetone (9: 1) as the solvent and the EtOAc–acetone (9: 1) eluate was recrystallized from acetone–hexane to give colorless amorphous HI (385 mg), mp 145–156\(^\circ\), Anal. Calcd. for C\(_{38}\)H\(_{56}\)O\(_{12}\): H\(_2\)O: C, 59.18; H, 5.48. Founded: C, 59.12; H, 5.34. [\(\alpha\)\(_D\)]\(^{25}\) \(-77.5\) (MeOH, \(c = 1.7\)). UV \(\lambda_{\text{max}}\) \(\text{nm}\) (log e): 230 (4.3), 244 (4.2), 253 (4.2), 300 (4.2), 320 (sh). \(R_{\text{f max}}\) cm\(^{-1}\): 3400 (OH), 1715 (C=O), 1650 (C=O). NMR (\(\delta\)–acetone) \(\delta\) ppm: 3.0–5.0 (CH\(_2\), CHOH, m), 8.38 (s, 3H, OH\(_3\)), 8.16, 8.90 (br. s. 2 OH, exchanged with D\(_2\)O) and the others as given in Table I.

The fraction A was purified by preparative TLC using the lower layer of CHCl\(_3\)–EtOH–H\(_2\)O (30: 7: 3) as the solvent to afford HI (108 mg) and I (5.6 mg). I, colorless amorphous (from acetone–hexane), mp 156–162\(^\circ\), Anal. Calcd. for C\(_{38}\)H\(_{56}\)O\(_{12}\): H\(_2\)O: C, 62.22; H, 5.52. Founded: C, 62.25; H, 5.59. [\(\alpha\)\(_D\)]\(^{25}\) \(-82.7\) (MeOH, \(c = 1.4\)). UV \(\lambda_{\text{max}}\) \(\text{nm}\) (log e): 228 (4.8), 244 (4.3), 253 (4.5), 300 (4.4). \(R_{\text{f max}}\) cm\(^{-1}\): 3400 (OH), 1715, (C=O), 1650 (C=O). NMR (\(\delta\)–DMSO) \(\delta\) ppm: 3.0–5.0 (CH\(_2\), CHOH, m), 9.95, 10.68 (br. s. 2 OH, exchanged with D\(_2\)O), and the others as given in Table I.

**Acid Hydrolysis of I Yielding Aloesin (III) and \(p\)-Coumaric Acid** — I (170 mg) was hydrolyzed with 10% HCl in dioxane–H\(_2\)O (1: 1) (50 ml) for 3 hr under reflux and after the evaporation of the solvent the residue (137 mg) was extracted with hot acetone (50 ml) three times. The extract was chromatographed over a silica gel column (10 g) using EtOAc and EtOAc–acetone (4: 1) as the solvent. The EtOAc eluate was recrystallized from EtOAc to give \(p\)-coumaric acid (12.3 mg), mp 202–204\(^\circ\), UV \(\lambda_{\text{max}}\) \(\text{nm}\): 225, 290, 310. \(R_{\text{f max}}\) cm\(^{-1}\): 3360 (OH), 1670 (C=O), 1630 (C=C), which was identified by the direct comparison (UV, IR, TLC and mixed melting point) with an authentic sample. The EtOAc–acetone (4: 1) eluate (93.5 mg) was chromatographed over a silica gel column (5 g) using EtOAc and EtOAc–acetone (4: 1) as the solvent and the EtOAc–acetone (4: 1) eluate (42 mg) was purified by preparative TLC (solvent 1 and 2) to give III, colorless amorphous (9 mg) (from acetone), mp 142–144\(^\circ\), UV \(\lambda_{\text{max}}\) \(\text{nm}\) (log e): 245 (4.4), 253 (4.4), 297 (4.3). \(R_{\text{f max}}\) cm\(^{-1}\): 3400 (OH), 1710 (C=O), 1650 (C=O). NMR (\(\delta\)–DMSO) \(\delta\) ppm: 3.2–4.8 (CH\(_2\), CHOH, m), 10.20 (s. OH, exchanged with D\(_2\)O) and the others as given in Table I, which was identified by the direct comparison (UV, IR, TLC and mixed melting point) with the authentic sample isolated from commercial aloesin (Merck).

Hydrolyses of II Yielding III and Ferulic Acid —a) Acid Hydrolysis: II (24 mg) was hydrolyzed with 10%, HCl–EtOH for 1 hr under reflux and after evaporation of the solvent the residue was chromatographed over a silica gel column (5 g) using CHCl₃ and EtOH by the gradient elution. The CHCl₃–EtOH (19:1) eluate afforded a spot of ferulic acid on TLC (solvent 1). The CHCl₃–EtOH (4:1) eluate was developed four times by preparative TLC (solvent 1) to give III, colorless amorphous (3 mg) (from acetone), mp 145—146°, which was identified by the direct comparison (UV, IR, TLC and mixed melting point) with an authentic sample.

b) Alkaline Hydrolysis: II (98 mg) was hydrolyzed with 2% Na₂CO₃ in EtOH–H₂O (1:1) for 1 hr under reflux and after neutralization of the solution the solvent was evaporated in vacuo. The residue was extracted with EtOAc and after evaporation of the solvent the residue was chromatographed over a silica gel column (5 g) using CHCl₃–EtOH as the solvent. The CHCl₃–EtOH (19:1) eluate gave ferulic acid, colorless needles (11 mg), mp 170—172°, which was identified by the direct comparison (UV, IR, TLC and mixed melting point) with an authentic sample.

Tritylation of I Yielding IV —To a solution of I (168 mg) dissolved in pyridine (7 ml) trityl chloride (227 mg) was added and after heating for 4 hr at 110°, the excess reagent was decomposed by adding H₂O, and then the reaction mixtures were extracted with ether. The extract (315 mg) was chromatographed over a silica gel column (15 g) using hexane–EtOAc (1:2) as the solvent. The hexane–EtOAc (1:2) eluate was recrystallized from EtOAc to give IV pale yellow amorphous (20 mg), mp 140—144°, Anal. Calcd. for C₁₅H₁₅O₂: C, 89.74; H, 5.60. Found: C, 89.66; H, 5.88. UV λmax nm (log e): 243 (4.4), 254 (4.3), 302 (4.4). IR νmax cm⁻¹: 3300 (OH), 1710 (C=O), 1650 (C=O), 700 (aromatic). NMR (D₂O) δ ppm: 3.5—5.0 (CH₃, CHO, m.), 7.2—7.6 (aromatic), 9.00 (s. 2 OH, exchanged with D₂O), and the others as given in Table I.

Tritylation of II Yielding V —To a solution of II (52 mg) dissolved in pyridine (2 ml) trityl chloride (108 mg) was added and the solution was heated for 4 hr at 100°. After working up in the same way as for IV, the crude product (129 mg) was chromatographed over a silica gel column (10 g) using hexane–EtOAc (1:2) by the gradient elution. The hexane–EtOAc eluate (24 mg) was purified by preparative TLC (solvent 3) to afford VI, pale yellow amorphous (13 mg), (from CHCl₃), mp 132—135°. Anal. Calcd. for C₂₆H₂₃O₁₄: 2H₂O: C, 67.91; H, 5.72. Found: C, 67.90; H, 5.70. UV λmax nm (log e): 228 (4.4), 244 (4.3), 253 (4.3), 298 (4.3). IR νmax cm⁻¹: 3400 (OH), 1720 (C=O), 1650 (C=O), 700 (aromatic). NMR (CDCl₃) δ ppm: 5.84 (s. 3H, CHO), 5.5—5.0 (CH₃, CHO, m.), 7.1—7.5 (aromatic), 9.00 (s. 2 OH, exchanged with D₂O), and the others as given in Table I.

Benzylidene Derivative (V) of I—To a solution of I (207 mg) dissolved in benzaldehyde (4 ml) anhydrous ZnCl₂ (1.4 g) was added and the reaction mixture was left stand for 24 hr at room temperature. The excess reagent was decomposed by adding ice H₂O and the precipitate was collected by filtration. The product was chromatographed over a silica gel column (10 g) using MeOH–CHCl₃ as the solvent. The CHCl₃–MeOH (49:1) eluate (54 mg) was further recrystallized from hexane–EtOAc (2:3) as the solvent. Finally, the fraction (25 mg) was recrystallized over a silica gel column (5 g) using CHCl₃–MeOH (49:1) as the solvent to yield VII, colorless amorphous (16 mg), (from CHCl₃), mp 218—222°. Anal. Calcd. for C₂₆H₂₃O₁₄: 2H₂O: C, 63.24; H, 5.46. Found: C, 63.70; H, 5.29. UV λmax nm (log e): 228 (4.4), 244 (4.3), 253 (4.3), 300 (4.3). IR νmax cm⁻¹: 3200 (OH), 1720 (C=O), 1650 (C=O), 700 (aromatic). NMR (DMSO) δ ppm: 3.5—5.2 (CH₂, CHO, m.), 5.64 (s. 1H, benzylidene methine), 7.2—7.6 (aromatic), 10.00, 10.08 (s. 2OH, exchanged with D₂O), and the others as given in Table I.

Benzylidene Derivative (VII) of II —To a solution of II (53 mg) dissolved in benzaldehyde (3 ml) anhydrous ZnCl₂ (1.1 g) was added and the reaction mixture was left stand for 5 hr at 80°. After working up in the same way as for V, the crude product was chromatographed over a silica gel column (5 g) using EtOH and CHCl₃ by the gradient elution. The CHCl₃–EtOH (20:1) eluate (21 mg) was purified by preparative TLC (solvent 3) to give VII, colorless amorphous (12 mg), (from CHCl₃), mp 188—191°. Anal. Calcd. for C₂₆H₂₃O₁₄: 2H₂O: C, 64.76; H, 5.30. Found: C, 64.34; H, 5.32. UV λmax nm (log e): 229 (4.4), 243 (4.3), 252 (4.2), 302 (4.3). IR νmax cm⁻¹: 3400 (OH), 1720 (C=O), 1650 (C=O), 700 (aromatic). NMR (CDCl₃) δ ppm: 3.88 (s. 3H, OCH₃), 5.72 (s. 1H, benzylidene methine), 3.4—5.4 (CH₂, CHO, m.), 7.2—7.6 (aromatic) and the others as given in Table I.

Acetylation of I Yielding Hexaacetate (Ia) —I (100 mg) was acetylated with Ac₂O–pyridine (1:3) (8 ml) at room temperature for 14 hr, followed by the usual work up. The product was chromatographed over a silica gel column (10 g) using hexane and EtOAc by the gradient elution. The hexane–EtOAc (2:3) eluate was recrystallized from hexane–CHCl₃ to afford Ia, colorless amorphous (23 mg), mp 124—125°. Anal. Calcd. for C₄₀H₄₀O₁₄: C, 69.62; H, 5.08. Found: C, 69.04; H, 5.08. UV λmax nm (log e): 226 (4.3), 252 (4.3), 292 (4.4). IR νmax cm⁻¹: 1760 (C=O), 1640 (C=O). NMR (CDCl₃) δ ppm: 3.8—5.5 (CH₃, CHOAc, m.), and the others as given in Table I.

Acetylation of II Yielding Hexaacetate (IIa) —II (51 mg) was acetylated with Ac₂O–pyridine (3:1) (4 ml) at room temperature for 14 hr, followed by the usual work up. The product was purified by preparative TLC using hexane–EtOAc (1:2) to give IIa, colorless amorphous (13 mg) (from hexane–CHCl₃), mp 118—120°. Anal. Calcd. for C₄₀H₄₀O₁₄: C, 59.85; H, 5.14. Found: C, 60.24; H, 5.28. UV λmax nm (log e): 250 (4.2), 296 (4.2). IR νmax cm⁻¹: 1750 (C=O), 1640 (C=O). NMR (CDCl₃) δ ppm: 3.81 (s. 3H, OCH₃), 3.8—5.5 (CH₂, CHOAc, m.), and the others as given in Table I.
Acetylation of III Yielding Hexacetate (IIIa)—III (110 mg) was acetylated with Ac₂O–pyridine (3:1) (8 ml) at room temperature for 14 hr, followed by the usual work up. After working up in the same way as for Ia, IIIa was recrystallized from CHCl₃–hexane to give IIIa, colorless needles (38 mg), mp 116–118°. UV λmax nm (log ε): 220 (4.1), 253 (4.2), 260 (4.2), 295 (4.1), 310 (sh.). IR ν/cm⁻¹: 1750 (C=O), 1640 (C=O).

Periodate Oxidation of I and II—To each solution of I or II (1/30—1/40 m mole) dissolved in MeOH (10 ml) and H₂O (5 ml), 0.02 N NaIO₄ aq. solution (5 ml) was added and the reaction mixture was left stand at 5° in darkness. As a blank test, 0.02 N NaIO₄ aq. solution (5 ml) without addition of sample was left stand in the same condition. At the definite time intervals 0.01 N solution of sodium arsenite (25 ml) and 2% KI solution (1 ml) were added to each solution. After keeping for 10 min, excess arsenite was titrated with 0.01 N I₂ solution. The results were given in Table II.

<table>
<thead>
<tr>
<th>Period of oxidation</th>
<th>Sample</th>
<th>10 min</th>
<th>1 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>0.83</td>
<td>0.92</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1.01</td>
<td>1.03</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, which is gratefully acknowledged. The authors express their thanks to Mr. Matsui and Miss Soeda for UV and IR spectral measurements, to Mr. Tanaka for NMR spectral measurement, to Mr. Ishimura for the microanalyses and to Miss Iwasa for her technical assistance. We are also grateful to Prof. H. Inouye of Kyoto University for his kind advice.