A New Antioxidative Glucoside Isolated from Oregano (*Origanum vulgare* L.)

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A new antioxidative glucoside (**1a**) was isolated from the leaves of oregano (*Origanum vulgare* L.), one of the herbal spices belonging to the Family Labiatae. The structure of **1a** was determined as 4-(3,4-dihydroxybenzoyloxymethyl)phenyl- β -D-glucopyranoside, on the basis of chemical and spectroscopic evidence. In addition, the structure was confirmed by synthesizing its acetyl derivative (**1b**).

Some spices are known to contain antioxidative components.^{1~6)} In our previous studies, several new antioxidative diterpenes were isolated from rosemary, one of the species of the Family Labiatae.^{7,8)}

Oregano (*Origanum vulgare* L.), belonging to the same family, has also been reported to show antioxidative activity.^{4~6)} Concerning the constituents of this plant, triterpenes,⁹⁾ phenolic acids¹⁰⁾ and volatile compounds¹¹⁾ have been detected only by the paper, thin-layer or gas chromatographic technique. Among these compounds, thymol and rosmarinic acid are known to have antioxidative activity.¹²⁾ However, to our knowledge, there have been few reports concerning the isolation of active compounds from oregano. In this paper, we describe the isolation and structural elucidation of a new antioxidative phenolic glucoside from oregano.

The dried leaves of oregano were successively extracted with dichloromethane and methanol. The methanol extract was partitioned between ethyl acetate and water, and the water-soluble fraction was subjected to chromatography on a polyamide column. The fraction eluted with 20% methanol in water gave compound **1a**, which was purified upon recrystallization from water to afford colorless needles, mp 204~205°C. Elementary analysis

and SIMS of this compound gave the molecular formula of C₂₀H₂₂O₁₀. The IR spectrum of compound **1a** showed hydroxyl groups in the region of 3500~3150 cm⁻¹, and a conjugated carbonyl group at 1680 cm⁻¹. The color reaction was positive to a ferric chloride solution. The ¹³C-NMR spectrum showed characteristic signals of a glucopyranosyl moiety at δ 102.1 (d), 74.8 (d), 78.1 (d), 71.3 (d), 77.9 (d) and 62.5 (t).¹³⁾ Other signals revealed the presence of one methylene (δ 67.0, t), one ester (δ 168.1, s), five substituted aromatic carbons (δ 158.9, 151.7, 146.1, 131.7 and 122.6, each a singlet) and seven protonated ones (δ 130.7 (2C), 123.7, 117.7 (2C), 117.4 and 115.8, each a doublet), which were attributed to the aglycone moiety. This spectral data indicates that compound **1a** had one glucose and two aromatic rings, one of which possessed two pairs of equivalent aromatic carbons. In addition, the ¹H-NMR spectrum showed protons of the A₂B₂ pattern at δ 7.11 (2H, d, J =8.8 Hz) and 7.37 (2H, d, J =8.8 Hz). These data support a 1,4-disubstituted benzene moiety being present in the molecule of compound **1a**. Proton signals of another aromatic ring were observed at δ 6.80 (1H, d) and 7.43 (2H, m). A two-proton singlet at δ 5.22 suggested the presence of a benzylic methylene bearing an acyloxy group.

Acetylation of compound **1a** with acetic anhydride and pyridine afforded a hexaacetate (**1b**), mp 58~59°C, whose structure is supported by the singlets at δ 2.31 for two phenolic acetates, and at δ 2.04, 2.05, 2.06 and 2.08 for four alcoholic acetates. The IR spectrum newly showed an absorption band of an acetate at 1750 cm⁻¹ with disappearance of the hydroxyl groups of **1a**. Two pairs of doublets at δ 7.00 (2H, d, J =8.6 Hz) and 7.37 (2H, d, J =8.6 Hz) confirmed the 1,4-disubstituted benzene moiety, and other aromatic proton signals at δ 7.28 (1H, d, J =8.5 Hz), 7.87 (1H, d, J =1.9 Hz) and 7.96 (1H, dd, J =1.9, 8.5 Hz) suggested 1,3,4-trisubstitution. The mass spectrum of **1b** showed a characteristic peak at m/z 331, derived from a terminal peracetylated hexose residue.¹⁴⁾ Furthermore, signals at δ 5.17 (1H, t, J =9.5 Hz), 5.25~5.35 (2H), 4.17 (1H, dd, J =2.4, 12.2 Hz) and 4.29 (1H, dd, J =5.4, 12.2 Hz) were attributed to the protons at 2', 3', 4' and 6' of tetraacetyl glucopyranoside, respectively. This fact supports the hexose being glucose, whose C-1' hydroxy group was substituted with an aglycone moiety. The stereochemistry of the anomeric proton was revealed to be β , based on a large coupling constant (J =7.3 Hz) in the ¹H-NMR spectrum of **1b** and a ¹³C-¹H coupling constant of 162 Hz in the ¹³C-NMR of **1a**.

The position of glucosidation on the aglycone was confirmed by methylation of **1a**, and subsequent alkaline hydrolysis. Methylation of **1a** with diazomethane gave a dimethyl derivative (**1c**), mp 134~135°C. In its ¹H-NMR spectrum, two singlets at δ 3.86 and 3.88 were attributed to phenolic methoxy groups. Alkaline hydrolysis of **1c** and subsequent acetylation gave 3,4-dimethoxybenzoic acid (**3b**) and 4-(tetra-*O*-acetyl- β -D-glucopyranosyloxy)benzyl acetate (**2b**). The spectral data of the latter compound were identical with those of an authentic sample¹⁵⁾ and of a synthetic one. This finding indicates compound **1a** to be an ester of 3,4-dihydroxybenzoic acid (**3a**) and 4-(β -D-glucopyranosyloxy)benzyl alcohol (**2a**).

Permethylation of **1a** by Hakomori's meth-

- 1a : R₁ = R₂ = H
 1b : R₁ = R₂ = COCH₃
 1c : R₁ = CH₃, R₂ = H

- 2a : R₁ = CH₂OH, R₂ = H
 2b : R₁ = CH₂OCOCH₃, R₂ = COCH₃
 2c : R₁ = CHO, R₂ = COCH₃
 2d : R₁ = CH₂OH, R₂ = COCH₃
 2e : R₁ = CH₂OCH₃, R₂ = CH₃

- 3a : R₁ = R₂ = H
 3b : R₁ = H, R₂ = CH₃
 3c : R₁ = R₂ = CH₃
 3d : R₁ = H, R₂ = COCH₃

od¹⁶⁾ gave methyl 3,4-dimethoxybenzoate (**3c**) and compound **2e**. In the ¹H-NMR spectrum of the latter compound (**2e**), four 3H singlets at δ 3.24, 3.32, 3.47 and 3.58 were assigned to the four methyl groups of the 2,3,4,6-tetra-*O*-methyl glucose moiety, which was supported by a characteristic fragment peak at m/z 218 as the base peak in its MS.¹⁷⁾ Two pairs of *ortho*-coupling protons were observed at δ 6.90 and 7.17, and a two-proton singlet at δ 4.30 was attributed to benzylic methylene protons. Thus, this compound (**2e**) was assigned to be 4-(tetra-*O*-methyl- β -D-glucopyranosyloxy)benzyl methyl ether, which was confirmed by comparing with the data of a synthetic specimen. This result also suggests that compound **1a** was an ester of 3,4-dihydroxybenzoic acid and 4-(β -D-glucopyranosyloxy)benzyl alcohol.

To confirm the structure of **1a**, its acetate (**1b**) was synthesized. Under the basic condition, *p*-hydroxybenzaldehyde was treated with tetra-*O*-acetyl- α -D-glucopyranosyl bromide,¹⁸⁾ mp 87~88°C, $[\alpha]_D^{19} +183^\circ$, to give

compound **2c**, mp 142~143°C, $[\alpha]_D^{20} -29.5^\circ$. In the IR spectrum, an absorption of a conjugated aldehyde was observed at 1692 cm^{-1} . Its $^1\text{H-NMR}$ spectrum showed the presence of a tetraacetyl glucose moiety, and of a *p*-substituted benzaldehyde moiety at δ 7.12 (2H, d, $J=8.2\text{ Hz}$), 7.88 (2H, d, $J=8.2\text{ Hz}$) and 9.96 (1H, s). Catalytic hydrogenation of **2c** with Pd-C in dioxane afforded compound **2d**, which was recrystallized from ethanol-water, mp 108~109°C, $[\alpha]_D^{24} -28.6^\circ$. Its IR spectrum newly showed the absorption band of a hydroxyl group at 3500 cm^{-1} , with disappearance of the formyl group at 1692 cm^{-1} . The signal of benzylic methylene protons bearing an oxygen atom was observed as a two-proton singlet at δ 4.66 in the $^1\text{H-NMR}$. Compound **2d** was esterified with 3,4-diacetoxybenzoic acid (**3d**) in the presence of dicyclohexylcarbodiimide to give compound **1b**. The spectral data of IR, $^1\text{H-NMR}$ and MS of this synthetic compound were identical with those of the acetyl derivative of compound **1a** in all respects. Thus it is concluded that the structure of compound **1a** was 4-(3,4-dihydroxybenzoyloxymethyl)phenyl- β -D-glucopyranoside.

The antioxidative activity of this new glucoside (**1a**) was measured by the ferric thiocyanate method in an aqueous ethanolic solution.¹⁹⁾ Compound **1a** showed stronger activity than α -tocopherol, and was comparable with that of BHA at a concentration of 0.01% (Fig. 1). This antioxidative glucoside might be useful as a natural antioxidant because of its solubility in water rather than in fat, whereas most other antioxidants are soluble in fat.

EXPERIMENTAL

Melting points (mp) were measured with a Yanagimoto micro-melting point apparatus and were uncorrected. UV absorption spectra were determined on a Hitachi 220 spectrophotometer, and IR spectra were recorded by a Jasco IR-S. $^1\text{H-NMR}$ (400.5 MHz) and $^{13}\text{C-NMR}$ (100.7 MHz) spectra were determined on a JEOL GX-400 using TMS as an internal standard. $^1\text{H-NMR}$ (60 MHz) spectra were run on a Hitachi R600. MS were obtained on a Shimadzu GCMS-7000S, and SIMS was determined on a Hitachi M-80B. Optical rotation was measured with an Union PM-101. Column chromatographies were performed using Wako polyamide C-200, Pharmacia Sephadex LH-20 and Merck silica gel 60 (70~230 mesh), and TLC was carried out using silica gel GF-254.

Extraction and fractionation. Dried ground leaves (1 kg) of oregano were successively extracted five times with CH_2Cl_2 (1.9 l each), and three times with MeOH (1.6 l each) at room temperature. The combined MeOH extract was concentrated on a rotary evaporator to yield a green mass (109 g). Ethyl acetate and water were added to the concentrate (34.1 g), and the mixture was stirred for 2 hr. The water-soluble part was freeze dried to afford a brownish powder (31.8 g). The freeze-dried sample (15 g) was dissolved in water and subjected to polyamide column chromatography, which was successively eluted with water, 20% MeOH aq. and MeOH. Finally, the column was eluted with a mixture of MeOH-HCOOH (995:5).

Isolation of compound 1a. The 20% MeOH aq. eluate was rechromatographed on a Sephadex LH-20 column with MeOH as the eluent to give compound **1a** (832 mg), which was recrystallized from water, mp 204~205°C. *Anal.* Found: C, 56.48; H, 5.16. Calcd. for $\text{C}_{20}\text{H}_{22}\text{O}_{10}$: C, 56.87; H, 5.25%. UV λ_{max} (MeOH) nm (log ϵ): 222 (4.49), 263 (3.13), 297 (2.86). $[\alpha]_D^{18} -112.1^\circ$ ($c=0.33$, MeOH). IR ν_{max} (Nujol) cm^{-1} : 3500~3150, 1680, 1610, 1515, 1235, 1045. $^1\text{H-NMR}$ (CD_3OD) δ : 3.35~3.50 (4H, m), 3.70 (1H, dd, $J=5.4, 12.1\text{ Hz}$), 3.90 (1H, dd, $J=2.1, 12.1\text{ Hz}$), 4.93 (1H, m), 5.22 (2H, s), 6.80 (1H, d, $J=8.1\text{ Hz}$), 7.11 (2H, d, $J=8.8\text{ Hz}$), 7.37 (2H, d, $J=8.8\text{ Hz}$), 7.43 (2H, m),

FIG. 1. Antioxidative Activity of Compound **1a** Measured by the Ferric Thiocyanate Method.

(Δ), conc. 0.01%; ($\bullet$), 0.02% ($\square$); BHA 0.01%; ($\blacksquare$), α -tocopherol 0.01%; (—), control.

^{13}C -NMR (CD_3OD) δ : 168.1 (s), 158.9 (s), 151.7 (s), 146.1 (s), 131.7 (s), 130.7 (d $\times$ 2), 123.7 (d), 122.6 (s), 117.7 (d $\times$ 2), 117.4 (d), 115.8 (d), 102.1 (d), 78.1 (d), 77.9 (d), 74.8 (d), 71.3 (d), 67.0 (t), 62.5 (t). SIMS m/z : 607 ($\text{M} + \text{H} + 2\text{G}$) $^+$, 515 ($\text{M} + \text{H} + \text{G}$) $^+$, 422 (M^+), 269.

Acetylation of compound 1a. To a solution of 100 mg of compound **1a** in pyridine (1 ml), acetic anhydride (1 ml) was added, and the mixture was allowed to stand overnight at room temperature. The reaction mixture was poured into cold 2N-HCl, and then extracted with benzene. The organic layer was washed with water, dried over anhydrous MgSO_4 and evaporated to dryness (99 mg). Recrystallization of this residue from CCl_4 gave colorless crystals of compound **1b**, mp 58–59°C. UV λ_{max} (EtOH) nm (log ϵ): 225 (4.21), 270 (3.11), 278 (2.97 sh). $[\alpha]_{\text{D}}^{19}$: -13.3° ($c=0.75$, CHCl_3). IR ν_{max} (Nujol) cm^{-1} : 1750, 1615, 1595, 1510, 1215. ^1H -NMR (CDCl_3) δ : 2.04, 2.05, 2.06, 2.08 (4 $\times$ 3H, s), 2.31 (6H, s), 3.87 (1H, ddd, $J=2.4, 5.4, 10.0$ Hz), 4.17 (1H, dd, $J=2.4, 12.2$ Hz), 4.29 (1H, dd, $J=5.4, 12.2$ Hz), 5.09 (1H, d, $J=7.3$ Hz), 5.17 (1H, t, $J=9.5$ Hz), 5.25–5.35 (2H, m), 5.29 (2H, s), 7.00 (2H, d, $J=8.6$ Hz), 7.28 (1H, d, $J=8.5$ Hz), 7.37 (2H, d, $J=8.6$ Hz), 7.87 (1H, d, $J=1.9$ Hz), 7.96 (1H, dd, $J=1.9, 8.5$ Hz). ^{13}C -NMR (CDCl_3) δ : 170.5 (s), 170.2 (s), 169.4 (s), 169.3 (s), 168.0 (s), 167.6 (s), 164.8 (s), 156.9 (s), 146.1 (s), 142.0 (s), 130.7 (s), 130.1 (d $\times$ 2), 128.6 (s), 128.2 (d), 125.1 (d), 123.5 (d), 117.0 (d $\times$ 2), 99.0 (d), 72.7 (d), 72.0 (d), 71.1 (d), 68.3 (d), 66.5 (t), 61.9 (t). MS m/z : 674 (M^+), 615, 452, 434, 331, 289, 271, 260, 229, 211, 196, 179, 169, 154, 137, 127, 109, 81.

Methylation of compound 1a. A solution of compound **1a** (100 mg) in MeOH (5 ml) was treated with ethereal diazomethane at 4°C, and the mixture was allowed to stand overnight at the same temperature. After the excess diazomethane had been decomposed with a few drops of acetic acid, the reaction mixture was concentrated *in vacuo* and the crude methylated product was recrystallized from acetone to give colorless crystals of compound **1c**, mp 134–135°C. UV λ_{max} (MeOH) nm (log ϵ): 221 (4.58), 262 (4.22), 292 (3.89). $[\alpha]_{\text{D}}^{20}$: -40.0° ($c=0.35$, MeOH). IR ν_{max} (Nujol) cm^{-1} : 3600–3100, 1710, 1600, 1510, 1270, 1225. ^1H -NMR (CD_3COCD_3) δ : 3.40–3.60 (4H, br. s), 3.86 (3H, s), 3.88 (3H, s), 4.90–5.10 (1H, m), 5.28 (2H, s), 7.04 (1H, d, $J=8.2$ Hz), 7.09 (2H, d, $J=9.4$ Hz), 7.44 (2H, d, $J=9.4$ Hz), 7.50–7.80 (2H, m).

Alkaline hydrolysis of compound 1c. Compound **1c** (39.4 mg) was dissolved in 2 ml of 3% methanolic KOH, and the mixture was left overnight at room temperature. After removing the solvent *in vacuo*, the reaction mixture was acetylated in the usual way. The acetylated material was poured into cold 2N-HCl, and subsequently extracted with ether. The organic layer was then extracted with aq. saturated NaHCO_3 solution.

The etherial layer was washed with water, dried over anhydrous MgSO_4 and concentrated. This neutral fraction was purified by silica gel column chromatography to give compound **2b**, which was recrystallized from methanol–water, mp 121–122°C. UV λ_{max} (EtOH) nm (log ϵ): 221 (3.99), 270 (2.91), 276 (2.86). $[\alpha]_{\text{D}}^{20}$: -20.6° ($c=0.68$, CHCl_3). IR ν_{max} (Nujol) cm^{-1} : 1750, 1725, 1610, 1590, 1512. ^1H -NMR (CDCl_3) δ : 2.03, 2.07 (15H, s), 3.93 (1H, m), 4.21 (2H, m), 5.05 (2H, s), 4.95–5.35 (4H, m), 6.98 (2H, d, $J=8.4$ Hz), 7.32 (2H, d, $J=8.4$ Hz).

The acidic fraction, which was obtained from the NaHCO_3 solution, was recrystallized from water to give compound **3b** (veratric acid). IR ν_{max} (Nujol) cm^{-1} : 3100–2400, 1675, 1590, 1515, 1270, 1235. ^1H -NMR (CD_3COCD_3) δ : 3.88 (3H, s), 3.90 (3H, s), 7.06 (1H, d, $J=8.2$ Hz), 7.50–7.80 (2H, m). MS m/z (%): 182 (M^+ , 100), 167 (40), 139 (20), 121 (29), 111 (34).

Permethylation of compound 1a by Hakomori's method. After washing mineral oil with dry hexane, NaH (0.4 g) was suspended in dimethylsulfoxide (DMSO, 6 ml) and stirred at 50°C for 2 hr under a nitrogen stream to give DMSO carbanion. A solution of compound **1a** (199 mg) in DMSO (1 ml) was added dropwise to the DMSO carbanion, and the mixture was stirred at room temperature for 30 min under a nitrogen stream, before adding CH_3I (2.3 g) with stirring at a temperature up to 25°C. The mixture was allowed to stand overnight in the same conditions. The reaction mixture was poured into a mixture of water and ice, and then extracted with ether. The organic layer was washed with water, dried over anhydrous MgSO_4 and filtered before the filtrate was concentrated to dryness. The residue was chromatographed on a silica gel column with a mixture of benzene–acetone as an eluent.

The first fraction eluted by benzene was recrystallized from hexane to afford compound **3c**, methyl 3,4-dimethoxybenzoate, mp 55–56°C. IR ν_{max} (Nujol) cm^{-1} : 1720, 1595, 1515, 1290, 1270, 1105, 1020. ^1H -NMR (CCl_4) δ : 3.86 (9H, s), 6.76 (1H, d, $J=8.2$ Hz), 7.40–7.70 (2H, m). MS m/z (%): 196 (M^+ , 100), 165 (98).

The second fraction eluted by benzene–acetone (95:5) was rechromatographed on a silica gel column and eluted with hexane–acetone (7:1) to give compound **2e** as a colorless oil. Anal. Found: C, 60.26; H, 7.77. Calcd. for $\text{C}_{18}\text{H}_{28}\text{O}_7$: C, 60.66; H, 7.92%. UV λ_{max} (EtOH) nm (log ϵ): 220 (4.03), 270 (3.28), 280 (3.15 sh). $[\alpha]_{\text{D}}^{19}$: -42.5° ($c=0.40$, CHCl_3). IR ν_{max} (film) cm^{-1} : 1610, 1510, 1230, 1150–1030. ^1H -NMR (CCl_4) δ : 3.0–3.8 (6H, m), 3.24 (3H, s), 3.32 (3H, s), 3.47 (3H, s), 3.58 (3H, s), 3.60 (3H, s), 4.30 (2H, s), 4.60–4.80 (1H, m), 6.90 (2H, d, $J=8.4$ Hz), 7.17 (2H, d, $J=8.4$ Hz). MS m/z : 356 (M^+), 218, 187, 155, 127, 111, 101.

Synthesis.

Compound 2c. *p*-Hydroxybenzaldehyde (3.4 g) was dis-

solved in a solution of 4% aqueous NaOH. To this mixture was added a solution of tetra-*O*-acetyl- α -D-glucopyranosyl bromide (mp 87~88°C, $[\alpha]_D^{19}$: +183°, 8.14 g) in acetone (57.4 ml). The mixture was allowed to stand for 5 hr with stirring at room temperature. Evaporation of the solvent gave a viscous residue, which was recrystallized from 95% EtOH to afford colorless needles of compound **2c** (2.4 g), mp 142~143°C. $[\alpha]_D^{20}$: -29.5° (c =0.44, CHCl₃). IR $\nu_{\max}$ (Nujol) cm⁻¹: 2750, 1735, 1692, 1600, 1505. ¹H-NMR (CDCl₃) δ : 2.05 (12H, s), 3.90~4.10 (1H, m), 4.23 (2H, m), 5.28 (4H, m), 7.12 (2H, d, J =8.2 Hz), 7.88 (2H, d, J =8.2 Hz), 9.96 (1H, s).

Compound 2d. A solution of compound **2c** (501 mg) in dioxane (20 ml) was stirred in a hydrogen atmosphere in the presence of 10% Pd-C (56 mg) as a catalyst at room temperature for 3 hr. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was subjected to silica gel column chromatography to give compound **2d**, which was recrystallized from ethanol-water, mp 108~109°C. $[\alpha]_D^{24}$: -28.6° (c =0.49, CHCl₃). IR $\nu_{\max}$ (Nujol) cm⁻¹: 3500, 3250, 1745, 1703, 1600, 1585, 1505. ¹H-NMR (CDCl₃) δ : 2.05, 2.07 (12H, s), 3.70~4.10 (1H, m), 4.21 (2H, m), 4.66 (2H, s), 5.0~5.4 (4H, m), 6.98 (2H, d, J =8.2 Hz), 7.33 (2H, d, J =8.2 Hz).

Compound 1b. Compound **2d** (279 mg) and 3,4-diacetoxybenzoic acid (221 mg) were mixed and dissolved in dry THF (20 ml), before adding dicyclohexylcarbodiimide (245 mg). The reaction mixture was stirred overnight at room temperature. The reaction product was concentrated *in vacuo* and to the residue was added acetone. The acetone-soluble material was purified by silica gel column chromatography to give compound **1b** (60 mg), which was recrystallized from CCl₄. The melting point, optical rotation and spectral data from IR and NMR of this compound were identical in all respects with those of the acetate (**1b**) derived from natural **1a**.

Compound 2b. To a solution of compound **2c** (1.0 g) in dioxane (20 ml) was added NaBH₄ (84 mg) in portions, and the mixture was stirred overnight at room temperature. After evaporating the solvent, the residue was treated overnight with acetic anhydride and pyridine at room temperature. The acetylated material was purified by silica gel column chromatography to give compound **2b**, which was recrystallized from methanol-water. The melting point, optical rotation and spectral data from IR and NMR were identical in all respects with those of authentic **2b**.

Compound 2e. Compound **2b** (211 mg) was dissolved in 6% methanolic KOH, and the mixture was allowed to stand overnight at room temperature. After evaporating the solvent, the residue was permethylated by Hakomori's method. The product was chromatographed on a silica gel column to give compound **2e** (45 mg) as a colorless oil. The spectral data from IR and NMR were identical in all respects with those of authentic **2e**.

Antioxidative assay (ferric thiocyanate method). One or two mg of samples were dissolved in 2 ml of 99.5% ethanol to give an assay solution of 0.01% or 0.02% at the final concentration. To each sample solution in a vial (ϕ =38, h =75 mm) with a screw cap, 2.052 ml of 2.51% linoleic acid in 99.5% ethanol, 4 ml of 0.05 M phosphate buffer (pH 7.0) and 1.948 ml of water were added, and then the vial was placed in an oven at 40°C in the dark. Every 24 hr, a 0.1 ml aliquot of this assay solution was diluted with 9.7 ml of 75% ethanol, which was followed by adding 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red color developed was measured at 500 nm.

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REFERENCES

- 1) D. J. Maveety, *U.S. Patent*, 2,124,706 (1938).
- 2) C. W. Dubois and D. K. Tressler, *Proc. Inst. Food Technol.*, **1943**, 202.
- 3) T. S. Ramaswamy and B. N. Banerjee, *Ann. Biochem. Exp. Med.*, **8**, 55 (1948).
- 4) J. R. Chipault, G. R. Mizuno, J. M. Hawakins and W. O. Lundberg, *Food Res.*, **17**, 46 (1952).
- 5) J. R. Chipault, G. R. Mizuno and W. O. Lundberg, *Food Technol.*, **10**, 209 (1956).
- 6) Y. Saito, U. Kimura and T. Sakamoto, *Eiyo and Shokuryo*, **29**, 404 (1976).
- 7) R. Inatani, N. Nakatani, H. Fuwa and H. Seto, *Agric. Biol. Chem.*, **46**, 1661 (1982).
- 8) N. Nakatani and R. Inatani, *Agric. Biol. Chem.*, **48**, 2081 (1984).
- 9) T. P. Pulatova, "Mater. Yubileinoi Resp. Nauchn. Konf. Farm," Posvyashch, 50-Letiye Obraz. (ed. by Khalmatov), SSSR Sep. 1972, p. 34 [*C. A.*, **82**, 167489 (1975)].
- 10) A. Reschke, *Z. Lebensm. Unters. Forsch.*, **176**, 116 (1983).
- 11) W. H. Stahl, J. N. Skarzynski and W. A. Voelker, *J. Ass. Offic. Anal. Chem.*, **52**, 1184 (1969) [*C. A.*, **72**, 30342 (1971)].
- 12) K. Herrmann, *Z. Lebensm. Unters. Forsch.*, **116**, 224 (1962).
- 13) P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, **53**, 1212 (1975).
- 14) I. A. Pearl and S. F. Darling, *Tetrahedron Lett.*, **20**, 1869 (1967).
- 15) H. Taguchi, I. Yoshioka, K. Yamasaki and H. Kim,

- Chem. Pharm. Bull.*, **29**, 55 (1981). 18) "Org. Syntheses," Coll. Vol. 3, p. 11.
- 16) S. Hakomori, *J. Biochem. (Tokyo)*, **55**, 205 (1964). 19) R. Inatani, N. Nakatani and H. Fuwa, *Agric. Biol. Chem.*, **47**, 521 (1983).
- 17) N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov and B. M. Zolotarev, *Tetrahedron*, **19**, 2209 (1963).
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