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Synthesis of Bredinin from 1-β-D-Ribofuranosyl-5-aminoimidazole-4-carboxamide by a Photo-Reaction

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Photolysis of 1-β-D-ribofuranosyl-5-aminoimidazole-4-carboxamide (AICA-riboside) gave 2-amino-*N*-(β-D-ribofuranosyl)malondiamide, which was cyclized by treatment with ethyl orthoformate to furnish 1-β-D-ribofuranosyl-5-hydroxyimidazole-4-carboxamide (bredinin), a potent immunosuppressive nucleoside antibiotic.

Keywords——nucleoside antibiotic; bredinin synthesis; AICA-riboside; photochemical reaction; anhydronucleoside; aminomalonamide; NMR

Bredinin (**1**) is an immunosuppressive and potent antitumor nucleoside antibiotic produced by *Eupenicillium brefeldianum* M-2166.[1-3] Its biological activities and the unique imidazole structure of the aglycone (4-carbamoylimidazolium-5-olate) make this compound an attractive target for synthetic studies. Hayashi and co-workers have synthesized bredinin by the glycosylation method using trimethylsilylated aglycone and tetra-*O*-acetylribose in the presence of a Lewis acid.[4] However, for a practical synthesis of bredinin, it is reasonable to select 1-β-D-ribofuranosyl-5-aminoimidazole-4-carboxamide (AICA-riboside) (**2**) as a starting material because of its commercial availability and the possibility of transforming the 5-amino function to the hydroxyl group.

This paper describes a conversion *via* a novel photochemical cleavage of the imidazole ring of AICA-riboside, followed by recyclization to **1**. A preliminary account of this work has appeared.[5]

It seemed that the diazotization of the 5-amino group of **2** was the simplest logical route to **1**. However, several attempts to diazotize **2** resulted in the formation of 2-azainosine as a main product, as reported by Kawana and co-workers.[6] In order to avoid the cyclization of the 5-diazonium intermediate, the 4-carbamoyl group of **2** was transformed to the nitrile group. Thus, **2** was treated with acetic anhydride in pyridine to give the 2′,3′,5′-tri-*O*-acetate (**3**), which was treated with phosphorus oxychloride and triethylamine to give the 4-cyano derivative (**4**).[7] Treatment of **4** with sodium nitrite in the presence of several kinds of acid and metal salts to stabilize the diazonium salt afforded a complex mixture (red colored) and a red precipitate. Instrumental investigations of the red precipitate showed that it was composed of an azo-coupled product (**5**). In fact, the spectral properties of **5** were similar to those of the known aglycone (**6**) of **5**.[8]

Next, the intramolecular attack of the 5′-hydroxyl group on the 5-diazonium function was attempted by taking account of the possibility of hydrolytic cleavage of the 5,5′-*O*-cyclolinkage thus formed. Compound **2** was converted to the 2′,3′-*O*-isopropylidene derivative, which was treated with phosphorus oxychloride to give the 4-cyano derivative (**7**). Treatment of **7** with nitrous acid afforded the 5,5′-anhydro derivative (**8**). However, the yield

of **8** was rather low and attempts to cleave the anhydro linkage of **8** were unsuccessful.

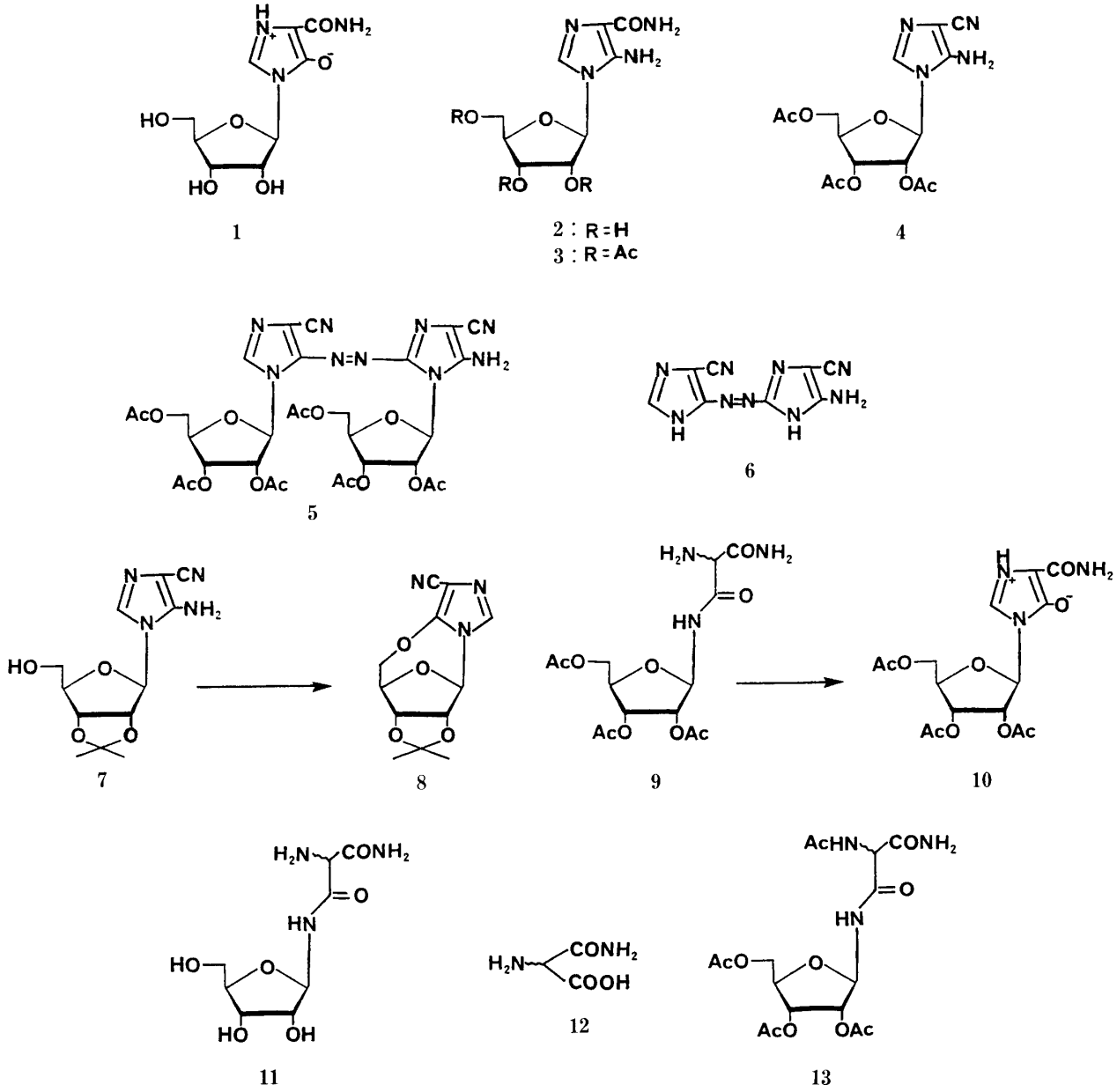


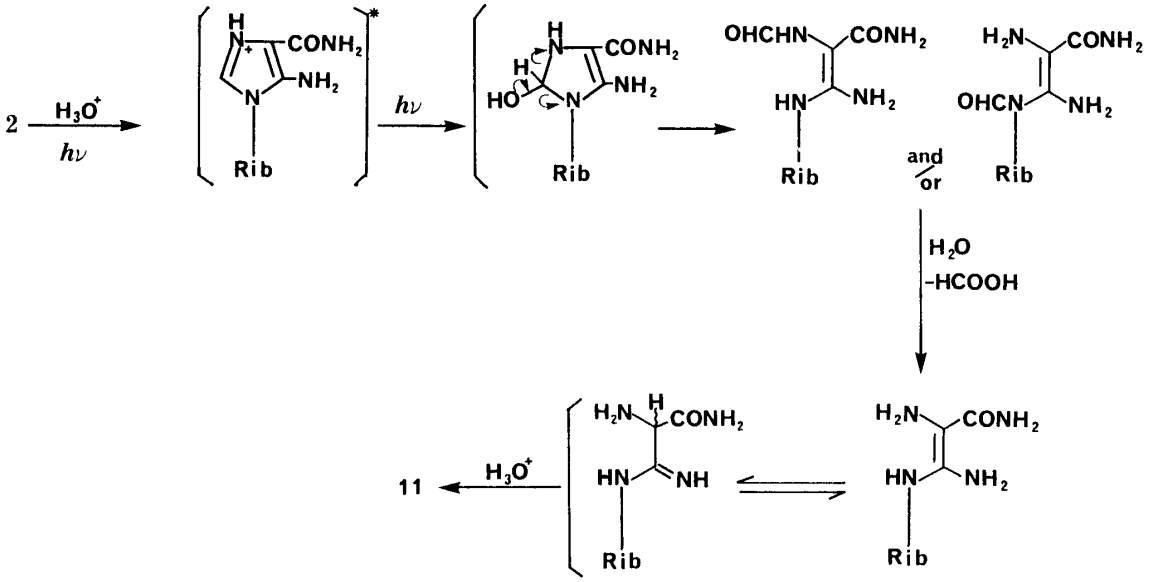
Chart 1

Recently, Stevens and Horton disclosed a synthesis of 4-carbamoyl-imidazolium-5-olate (the aglycone of bredinin) from 5-diazoimidazole-4-carboxamide by photolysis under acidic conditions.[8] After several experiments on the photolysis of imidazole derivatives, we achieved the direct ring cleavage of AICA derivatives by photoirradiation. Irradiation of tri-*O*-acetyl-AICA-riboside (**3**) in 0.02 N hydrochloric acid with a high-pressure mercury lamp (400 W) through a Pyrex filter for 15 h afforded several products. Among them, 2-amino-*N*-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)malondiamide (**9**) was obtained after separation by column chromatography on silica gel. The mass spectrum (MS) of **9** showed the MH^+ ion peak at *m/z* 276 (CI-isoBu) and the proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed the presence of a set of signals, suggesting that **9** is an epimeric mixture. Fractional crystallization of **9** from methanol gave one epimer (**9a**) as crystals and the other (**9b**) as a foam. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra showed the C-3 carbon

at 58.7 ppm (doublet) and 58.5 ppm (doublet) for **9a** and **9b**, respectively. Treatment of **9** with ethyl orthoformate in dimethylformamide (DMF) afforded 2′,3′,5′-tri-*O*-acetylbredinin (**10**; identical with an authentic sample) in 75% yield, and deacetylation of **10** in methanolic ammonia furnished bredinin (**1**).

A similar photoreaction and recyclization were carried out from **2**. Thus, photolysis of **2** in acidic medium gave the ribosylaminomalondiamide (**11**), which was identical with the compound obtained by the deacetylation of **9**. The de-glycosylated product, aminomalonic acid monoamide (**12**),[9] was also isolated and it was further converted to glycinamide[9] on heating of the aqueous solution. Acetylation of **9** and **11** afforded the same tetra-acetate (**13**). Recyclization of **11** with ethyl orthoformate afforded bredinin (**1**) in 11.2% overall yield after recrystallization from aqueous isopropanol. The synthetic sample had the same physical and biological properties as an authentic sample.

Although a mechanistic study of this novel photolysis of AICA-riboside was not attempted, the reaction should proceed by the photo-induced hydrolysis of protonated **2** at the C-2 position as depicted in Chart 2.



Rib = β-D-ribofuranosyl

Chart 2

Experimental

Melting points were determined on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded with a JEOL FX-100-FT spectrometer in $CDCl_3$ or DMSO-d_6 as the solvent, with tetramethylsilane as an internal standard. Chemical shifts are reported in ppm (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), br (broad) or m (multiplet). All exchangeable protons were confirmed by addition of D_2O. Ultraviolet spectra (UV) were recorded with a Shimadzu UV-250 spectrophotometer and infrared spectra (IR) with a Hitachi 260-50 spectrophotometer. Specific rotations were measured on a Horiba SEPA-200 polarimeter. MS were measured on a JEOL D-300 (EI or CI) spectrometer. Thin layer chromatography (TLC) was carried out on Merck pre-coated plates 60 F_{254}, and silica gel preparative-TLC (PTLC) and column chromatography were performed on Wako-gel C-200.

5-Amino-4-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (4)——Compound **4** was prepared essentially by the reported method.[7] Acetic anhydride (66 ml) was added dropwise to an ice-cooled suspension of **2** (20 g) in dry pyridine (240 ml). The mixture was stirred at room temperature for 40 min to give a clear solution. After 1.5 h, the reaction mixture was cooled in an ice-bath and MeOH (100 ml) was added. The solvent was removed *in vacuo* and the residue was partitioned between CH_2Cl_2 and H_2O (300 ml each). The organic layer was passed through a phase separating paper (Whatman 1PS) and the filtrate was concentrated to give the 2′,3′,5′-tri-*O*-acetate (**3**) of **2** as a foam

in quantitave yield. This sample was used for the next step without purification. ^{1}H-NMR ($CDCl_3$): 7.17 (1H, s, H-2), 5.70 (1H, d, H-1′), 5.6—5.3 (4H, m, H-2′, 3′ and H_2N), 4.42 (3H, m, H-4′, 5′), 2.14, 2.13, 2.12 (9H, each s, AcO). MS *m/z*: 384 (M^+). Compound **3** (12.52 g) and triethylamine (22.7 ml) were dissolved in $CHCl_3$ (250 ml) under cooling in an ice-bath. Phosphorus oxychloride (3.3 ml) was added dropwise with stirring over a period of 1.5 h and the solution was stirred at 0 °C for an additional 1 h. The mixture was poured into ice-water with vigorous stirring and the organic layer was separated, then the aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with H_2O, 0.05 N HCl, and H_2O, and passed through a Whatman 1PS filter paper. The filtrate was concentrated *in vacuo* and the residue was taken up in $CHCl_3$ and applied to a column of silica gel. The eluate with $CHCl_3$–MeOH (5 : 1) was concentrated to leave **4** (7.30 g, 62%) as a foam. MS *m/z*: 366 (M^+). IR (neat): 2200 cm^{-1} (ν_{CN}). ^{1}H-NMR ($CDCl_3$): 7.27 (1H, s, H-2), 5.68 (1H, d, H-1′), 5.44 (1H, dd, H-2′), 5.32 (1H, dd, H-3′), 4.87 (2H, br, H_2N), 4.41 (3H, m, H-4′, 5′), 2.15 (9H, s, AcO × 3).

Reaction of 4 with Nitrous Acid——Compound **4** (1.1 g in 2 ml of MeOH and 15 ml of 1 N HCl) was added to an ice-cold solution of $NaNO_2$ (330 mg) and $SnCl_4 \cdot xH_2O$ (500 mg) in H_2O. Precipitated red material was collected by filtration and purified through a silica gel column ($CHCl_3$–MeOH, 30 : 1) to give product **5** (274 mg). UV λ_{max}^{MeOH} nm: 522. IR: 2230 cm^{-1} (ν_{CN}). ^{1}H-NMR ($CDCl_3$): 8.78 (1H, s, H-2), 6.38, 6.76 (1H each, d, H-1′ and H-1″), 5.75 (2H, br, H_2N), 5.7—5.2 (4H, m, H-2′, 2″, 3′, 3″), 4.8—4.2 (6H, m, H-4′, 4″, 5′, 5″), 2.2—2.0 (18H, Ac × 6).

5-Amino-4-cyano-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (7)——2′,3′-*O*-Isopropylidene-AICA riboside (298 mg) and triethylamine (695 μl) were dissolved in dry tetrahydrofuran (THF) (8 ml). $POCl_3$ (100 μl) was added to this solution under ice-cooling, and the mixture was stirred for 5 h at room temperature. A small amount of aqueous $NaHCO_3$ was added to the solution and the solvent was removed *in vacuo*. The residue was extracted with EtOAc several times and the organic layer was washed with H_2O, passed through Whatman 1PS filter paper, and evaporated *in vacuo*. The residue was subjected to PTLC ($CHCl_3$–MeOH, 7 : 1). The appropriate band was extracted with $CHCl_3$–EtOH (1 : 1) and the solvent was evaporated off to leave **7** (125 mg, 45%), mp 188—190 °C. MS (CI-isoBu) *m/z*: 281 (MH^+). IR (KBr): 2220 cm^{-1} (ν_{CN}). ^{1}H-NMR ($CDCl_3$): 7.46 (1H, s, H-2), 6.38 (2H, br, H_2N), 5.76 (1H, d, H-1′), 5.29 (1H, t, H-5′), 5.04 (1H, dd, H-2′), 4.86 (1H, dd, H-3′), 4.14 (1H, m, H-4′), 3.52 (2H, m, H-5′), 1.53, 1.31 (3H each, s, Me_2C).

5,5′-Anhydro-4-cyano-5-hydroxy-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (8)——$NaNO_2$ (56 mg) was dissolved in H_2O (2 ml), and AcOH (0.1 ml) was added to the resultant solution in an ice-bath. Next, a 10% AcOH solution of **7** (112 mg) was added dropwise over a period of 30 min. The mixture was stirred for 1 h and the solution was concentrated *in vacuo*. The residue was subjected to PTLC (AcOEt–$CHCl_3$, 2 : 3). From the extract of the appropriate band with $CHCl_3$–EtOH (1 : 1), **8** (19 mg, 18%) was obtained as a foam. UV λ_{max}^{MeOH} nm: 300. MS (CI-isoBu) *m/z*: 264 (MH^+). ^{1}H-NMR (DMSO-d_6): 8.48 (1H, s, H-2), 6.39 (1H, s, H-1′), 5.16 (1H, d, H-2′), 4.80 (1H, dd, H-3′), 4.38 (1H, q, H-4′), 3.70 (1H, dd, H-5′a, $J_{a,b}$ = 12 Hz), 3.54 (1H, dd, H-5′b), 1.51, 1.30 (3H each, s, Me_2C).

2-Amino-*N*-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)malondiamide (9)——A solution of **3** (384 mg) in 0.02 N HCl (360 ml) was irradiated with a 400 W Hg lamp for 15 h through a Pyrex filter while argon was bubbled through. The solution was neutralized by addition of Dowex 1 (OH^-) resin. The resin was filtered off and the filtrate was evaporated *in vacuo*. The residue was partitioned between $CHCl_3$ and H_2O, and the organic layer was separated. The solvent was evaporated off and the residue was subjected to a flash column silica gel chromatography. The eluate with $CHCl_3$–MeOH (17 : 1) was concentrated to leave an epimeric mixture of **9** (120 mg, 32%) as a foam. MS (CI-isoBu) *m/z*: 376 (MH^+). $[\alpha]_D^{24}$: −14.3° (*c* = 0.5, $CHCl_3$). Crystallization of **9** from MeOH gave **9a**. Compound **9b** was obtained as foam.

Physical Constants of **9a**: mp 122—124 °C. $[\alpha]_D^{24}$: −39.8° (*c* = 0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, D_2O): 5.67 (1H, d, H-1′, *J* = 5.0 Hz), 2.15 (3H, s, AcO), 2.09 (6H, s, AcO × 2). ^{13}C-NMR (DMSO-d_6): 20.09, 20.58, 58.67 (d, C-3), 63.37 (t, C-5′), 70.17 (d, C-3′), 72.99 (d, C-2′), 77.72 (d, C-4′), 81.76 (d, C-1′), 169.34, 170.09, 171.18, 171.42. *Anal.* Calcd for $C_{14}H_{21}N_3O_9$: C, 44.80; H, 5.64; N, 11.20. Found: C, 45.04; H, 5.73; N, 10.81.

Physical Constants of **9b**: $[\alpha]_D^{24}$: −6.9° (*c* = 0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, D_2O): 5.62 (1H, d, H-1′, *J* = 3.0 Hz), 5.28 (2H, m, H-2′, 3′), 4.24 (4H, m, H-3, 4′, 5′), 2.14 (3H, s, AcO), 2.09 (6H, s, AcO × 2). ^{13}C-NMR (DMSO-d_6): 20.35, 20.64, 58.46 (d, C-3), 63.42 (t, C-5′), 70.23 (d, C-3′), 73.05 (d, C-2′), 77.78 (d, C-4′), 81.81 (d, C-1′), 169.39, 170.14, 170.69, 170.83.

2′,3′,5′-Tri-*O*-acetylbredinin (10)——A mixture of **9** (anomeric mixture, 960 mg) and ethyl orthoformate (554 μl, 1.3 eq) in dry DMF (25 ml) was stirred at 100 °C for 10 min, then at 110 °C for 20 min. The solvent was removed *in vacuo* and the residue was applied to a flash column of silica gel. The eluate with $CHCl_3$–MeOH (10 : 1) was concentrated and the residue was crystallized from MeOH to give **10** (736 mg, 75%), mp 184—186 °C (authentic bredinin triacetate, mp 188—190 °C). UV λ_{max}^{MeOH} nm: 284, 243. $\lambda_{max}^{0.1\ N NaOH}$ nm: 276. IR (KBr): 1740, 1670, 1640, 1600, 1560, 1440, 1380, 1240, 1110, 1060 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 8.35 (1H, s, H-2), 6.88 (2H, br, NH_2), 5.80 (2H, m, H-1′, 2′), 5.52 (1H, m, H-3′), 4.4—4.1 (3H, m, H-4′, 5′), 2.08, 2.07, 2.03 (3H, each s, Ac × 3). *Anal.* Calcd for $C_{15}H_{19}N_3O_9$: C, 46.70; H, 4.97; N, 10.90. Found: C, 46.86; H, 4.73; N, 11.43. These spectroscopic data were consistent with those of **10** obtained by acetylation of bredinin.

Bredinin (1) from 10——Compound **10** (510 mg) was dissolved in MeOH–NH_3 (20 ml) and kept at room temperature for 5.5 h. The solvent was removed *in vacuo* and the residue was dissolved in hot MeOH. *n*-PrOH was

added to the solution and cooled. The precipitated crystals of **1** (283 mg, 82%) were collected. This sample was identical with an authentic specimen on the basis of the following spectroscopic data. UV $\lambda_{max}^{H_2O}$ nm: 277, 243. $\lambda_{max}^{0.1\,N\,HCl}$ nm: 278, 243. $\lambda_{max}^{0.1\,N\,NaOH}$ nm: 275. ^{1}H-NMR (DMSO-d_6): 8.31 (1H, s, H-2), 6.90 (2H, br d, NH_2), 5.52 (1H, d, H-1′, J=5.4 Hz), 6.0—4.8 (3H, br, OH-2′, 3′, 5′), 4.39 (1H, t, H-2′), 4.06 (1H, t, H-3′), 3.90 (1H, m, H-4′), 3.58 (2H, m, H-5′). The *in vitro* growth inhibitory activities of this compound and natural bredinin were tested against cultured L5178Y cells (derived from murine lymphoma). Both compounds inhibited the cell growth completely at a concentration of 5 μg/ml when they were added to 10^4 cells/ml of cell culture and incubated at 37 °C for 45 h.

2-Amino-*N*-(β-D-ribofuranosyl)malondiamide (11) and Aminomalonic Monoamide (12) from 2 by Photolysis——A solution of **2** (516 mg, 2 mmol) in 0.02 N HCl (500 ml) was irradiated for 6 h. The solution was neutrallized by addition of Dowex 1 (OH^-) and the resin was filtered off. The filtrate was evaporated to leave **11** as a foam. The separated resin was placed in a column and eluted with H_2O, then with 2% HCO_2H. The latter eluate was evaporated and the residue was crystallized from 90% MeOH to give **12** (68 mg), mp 111—112 °C (lit., 121—122 °C).[9] IR (KBr): 1680 cm^{-1} (ν_{CO}). ^{1}H-NMR (D_2O): 4.77 (s). *Anal.* Calcd for $C_3H_6N_2O_3$: C, 30.51; H, 5.12; N, 23.72. Found: C, 30.63; H, 5.15; N, 24.92. An aqueous solution of **12** was heated to give glycinamide as described by Izumiya *et al.*[9]

2-Amino-*N*-(β-D-ribofuranosyl)malondiamide (11) by Ammonolysis of 9——A solution of **9** (375 mg) in methanolic ammonia (15 ml) was kept at room temperature for 12 h. The solvent was removed *in vacuo* and the residue was dissolved in H_2O and applied to a column of Dowex 50 (H^+). The column was washed with H_2O, then **11** was eluted with 0.1 N NH_4OH. The solvent was evaporated off to leave **11** (208 mg) as a foam. ^{1}H-NMR (DMSO-d_6, D_2O): 5.20 (1H, d, H-1′), 3.92—3.50 (5H, m, H-2′, 3′, 4′, 5′).

2-Acetamido-*N*-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)malondiamide (13) from 9 or 11——a) Acetic anhydride (0.2 ml) was added to a solution of **9** (320 mg) in pyridine (5 ml) under cooling in an ice-bath, and the mixture was stirred for 2 h at room temperature. MeOH was added, the solvent was removed *in vacuo*, and the residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH (19 : 1) to give **13** (324 mg, 91%) as a foam. Physical constants of **13** were identical with those of the product obtained by method b). b) Acetic anhydride (1.0 ml) was added to a solution of **11** (obtained from 516 mg of **2** by photo-irradiation) in pyridine (5 ml), and the mixture was stirred for 2 h at room temperature. The solvent was removed *in vacuo* and the residue was applied to a column of silica gel. The eluate with $CHCl_3$–MeOH (19 : 1) was concentrated to leave **13** (106 mg, 13% from **2**) as a foam. MS (CI-isoBu) *m/z*: 418 (MH^+). ^{1}H-NMR ($CDCl_3$–D_2O): 5.63 (1H, d+d, H-1′), 5.29 (2H, m, H-2′, 3′), 5.07, 5.03 (1H total, s, H-2), 4.22 (3H, m, H-4′, 5′), 2.09 (12H, Ac×4).

Bredinin (1) from 2——A solution of **2** (1.55 g, 6 mmol) in 0.02 N HCl (500 ml) was irradiated for 15 h. The solution was made slightly alkaline by addition of Dowex 1 (OH^-). The resin was filtered off, the filtrate was concentrated, and the residual foam (**11**) was dried *in vacuo* for 5 h at 40 °C. This foam was dissolved in DMF (20 ml) with ethyl orthoformate (0.4 ml) and the solution was heated at 130 °C for 7 min. The reaction mixture was applied to a column (2×15 cm) of IRA-411 (OH^-) and the column was washed with H_2O, then eluted with 2% AcOH. The eluate was concentrated *in vacuo* and the residue was subjected to PTLC (developed with AcOBu–acetone–H_2O–AcOH (10 : 3 : 4 : 6)). The appropriate band was eluted with H_2O and the solvent was removed *in vacuo*. The residue was dissolved in H_2O and passed through a column of Dowex 50 (H^+) for decolorization. The eluate was concentrated and the residue was crystallized from H_2O–iso-PrOH to give **1** (174 mg, 11.3% from **2**). The physical constants were identical with those of an authentic specimen.

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