NEW BRIARANE DITERPENES FROM A GORGONACEAN *BRIAREUM* SP.

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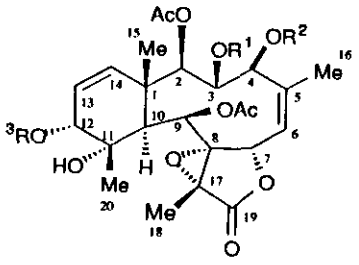
Abstract- Two new diterpenes possessing a 2,3,4-oxidized briarane skeleton have been isolated from a gorgonacean *Briareum* sp.

The genus *Briareum* (subclass Alcyonaria, order Goargonaceae, family Briareidae) has produced a number of briarane type diterpenes, containing a γ-lactone in a bicyclo [8.4.0] system.[1] The majority of these diterpenes showed interesting biological activity such as cytotoxic, anti-flammatory, and antiviral activity.[2] We examined bioactive constituents of a *Briareum* sp., formerly classified as *Pachyclavularia violacea* of the soft coral. The methanol extract of the *Briareum* sp., collected in the area of Bonotsu, Kagoshima prefecture, was partitioned between dichloromethane and water. Bioassay guided fractionation of the dichloromethane extract, which showed ichthyotoxity against Japanese killifish, *Orzia latipes*, by a combination of silica gel column chromatography and HPLC gave two new briarane derivatives (**1**) and (**2**).

Compound (**1**), $C_{32}H_{46}O_{12}$, showed IR absorption bands of a hydroxy group (3443 cm^{-1}), a γ-lactone (1784 cm^{-1}), and an ester carbonyl (1741 cm^{-1}). The molecular formula indicated ten degrees of unsaturation. Four olefinic resonances [δ_C 124.2 (d), 125.1 (d), 137.9 (d), and 141.0 (s)] and four ester carbon resonances [δ_C 168.4, 169.6, 170.5, and 173.5 (each, s) in the ^{13}C NMR spectrum accounted for six double bond equivalents, suggesting that **1** is a tetracyclic compound. The ^1H and ^{13}C spectra and ^{13}C-^1H COSY indicated the presence of seven methyl groups: one tertiary methyl [δ_H 1.32 (3H, s), δ_C 15.5 (s)], two tertiary methyls on a carbon carrying an oxygen function [δ_H 1.16 (3H, s), δ_C 21.4 (s) and 1.70 (3H, s), δ_H 9.7 (s)], a vinyl methyl [δ_H 2.08 (3H, d, J=1.1 Hz), δ_C 25.5 (s)], two acetyl methyls [δ_H 2.22 (3H, s), 20.9 (s)] and δ_H 2.27 [(3H, s), δ_C 21.4 (s)], and a primary methyl due to a *n*-octanoate moiety [δ_H 0.86 (3H, t, J=7.0 Hz, H-28), δ_C 14.1 (q); δ_H *ca.* 1.29 (8H, m, CH_2 x 4, H-24, H-25, H-26, and H-27), δ_C 22.6, 28.9, 29.0, 31.6 (each t); δ_H 1.63 (2H, m, H-23), δ_C 24.9 (t), and δ_H *ca.* 2.38 (2H, m, H-22), δ_C 34.3 (t), and δ_C 173.5 (s, C-21)].[3] Subtraction of the 12 carbon atoms associated with the three ester moieties from 32 carbon atoms in **1** left 20 carbon atoms, implying that **1** would be a diterpene.



1 R^1=H, R^2=$CO(CH_2)_6Me$, R^3=H

2 R^1=Ac, R^2=Ac, R^3=H

3 R^1=Ac, R^2=$CO(CH_2)_6Me$, R^3=Ac

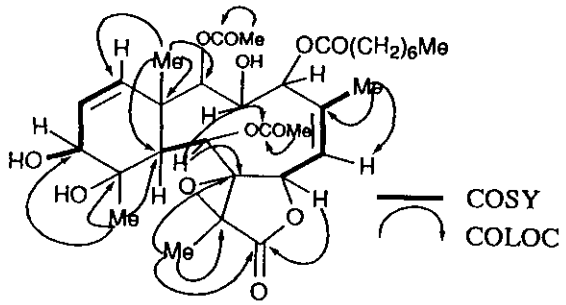


Figure 1. ^{1}H-^{1}H COSY and COLOC correlations of **1**

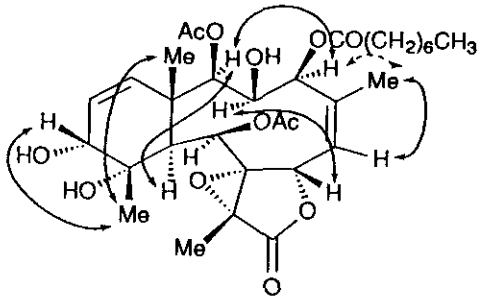


Figure 2. NOE correlations of **1**.

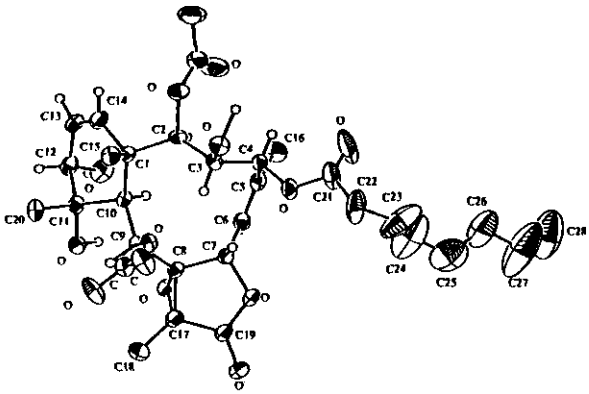


Figure 3. ORTEP representation of **1**.

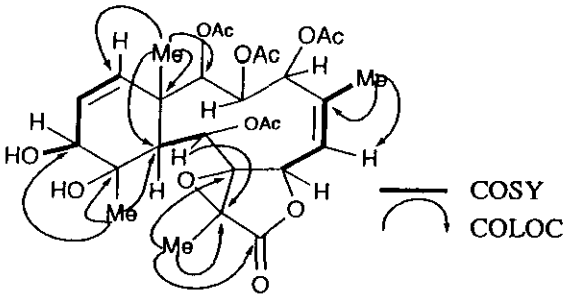


Figure 4. ^{1}H-^{1}H COSY and COLOC correlations of **2**.

The ^{1}H-^{1}H COSY correlations resolved three isolated proton spin systems (Figure 1). H-6 [δ 5.50 (1H, br d, J=9.5 Hz)] was coupled to H-7 [δ 5.72 (1H, d, J=9.5 Hz] and weakly to H-16 (δ_H 2.08). H-9 [δ 5.92 (1H, d, 3.8 Hz)] was coupled to H-10 [δ 2.58 (3H, d, J=3.8 Hz)]. H-12 [δ 3.69 (1H, t, J=6.2 Hz)] was coupled to a hydroxy proton [δ_H *ca.* 2.38 (1H, overlapped)] and H-13 [δ_H 5.82 (1H, dd, J=6.2 and 10.4 Hz)], the latter of which in turn was coupled to H-14 [(δ 5.35, 1H, d, J=10.4 Hz)]. Three resonances at δ 4.70 (1H, br s), 4.86 (1H, overlapped), and 4.87 (1H, overlapped) were not correlated to each other. The large part of the gross structure was completed by COLOC experiments as shown in Figure1. The connectivity of C-1 to C-10 and C-14 resulted from cross peaks from H-15 (δ_H 1.32, 3H, s) to C-1 (δ_C 47.1, s), C-10 (δ_C 43.8, d), and C-14 (δ_C 137.9, d). The linkage from C-10 to C-12 was

suggested by cross peaks from H-20 (δ_H 1.16) to C-10, C-11 (δ_C 73.7, s), and C-12 (δ_C 70,2, d). Therefore the above results indicated the presence of a six-membered ring. Correlations were observed from H-9 to C-8, and C-9 acetoxyl carbonyl (δ_C 168.4) which also showed a cross peak with the acetoxy methyl protons (δ_H 2.27s). The chemical shift of the remaining acetoxy carbonyl was determined to be δ_C 169.6 from the correlation of acetoxy methyl protons (δ_H 2.22, 3H, s) with the carbonyl. Therefore the chemical shift of the γ-lactone carbonyl carbon was concluded to be δ_C 170.5. H-18 (δ_H 1.70, s) showed correlations to C-8, C-17 (δ_C 65.5, s), and the γ-lactone carbonyl carbon at C-19, suggesting an α-methyl γ-lactone. A Correlation was also observed between H-7 and the γ-lactone carbonyl carbon. Acetylation of **1** with acetic anhydride in pyridine yielded a tetraacetate (**3**), $C_{36}H_{50}O_{14}$, the IR spectra of which showed absorption band due to a tertiary hydroxy group (3549 cm^{-1}). Thus, 11 of 12 oxygen atoms in **1** was accounted for. These oxygen atoms were due to three acyl moieties, three hydroxy groups, and a γ-lactone. Based on the results, compound (**1**) was assumed to possess the same briarane skeleton as briarane A which had been isolated from the gorgonacean *Briareum asbestinum*.[4] The presence of an epoxide as the twelfth oxygen atom and the fourth ring between C-8 and C-17 was therefore deduced from the chemical shifts of C-8 (δ_C 71.3) and C-17 (δ_C 64.5), which also meant that the tertiary hydroxy group was located at C-11. The remaining problem was to determine the positions of two hydroxy group, an acetoxyl group, and a *n*-octanoate group at C-2, C-3, C-4, or C-12. The two hydroxy groups were concluded to be placed at C-3 and C-12, since the H-3 and the H-12 in **1** were shifted to downfield (Δ 1.22 ppm and Δ 0.98 ppm, respectively) after acetylation. In the COLOC experiments of **1** in C_5D_5N, H-16 (δ_H 2.29, 3H, br s) showed a cross peak with C-4 (δ_C 78.4, d) as well as the cross peak between H-15 (δ_H 1.73) and C-2 (δ_C 78.4, d). Thus, the acetoxyl group and the *n*-octanoate group could be placed at C-2 or C-4, and the remaining hydroxy group at C-3.

The relative stereochemistry of all chiral centers was elucidated from NOE experiments of **1** (Figure. 2) and the proton-proton coupling constants of **3**. NOEs between H-20 and H-15 and H-12 showed that these protons occur on the same face of the ring system (β) and the ring junction is *trans*. An NOE between H-2 and H-10 suggested that they were on the opposite face (α) to H-15. In the ^1H NMR spectrum of the acetate (**3**), signals due to H-2 appeared as a broad singlet at δ_H 5.02, while signals due to H-3 and H-4 were found as a double doublet (J=1.6 and 10.3 Hz) at δ_H 6.61 and a broad doublet (J=10.3 Hz) at δ_H 5.69, respectively. This suggested that H-2 and H-3 are orthogonal to each other and H-3 and H-4 are antiparallel. NOEs from H-4 to H-2 and H-16, from H-6 to H-16, and from H-3 to H-7 were observed. On the above results, it was concluded that H-3 and H-4 were α-oriented and H-7 was β-oriented, and H-6 and H-15 were folded downward as for other briarane derivatives.[5] The *Z* nature of the double bond at C-5 was confirmed on the basis of the NOE between H-6 and H-16 and the C-16 chemical shift (δ_C 25.5). Configuration of H-9 was deduced to be α from the observation of a NOE between H-9 and H-18. To determine the positions of the acetoxyl group and *n*-octanoate moiety at C-2 or C-4, the stereochemistry of the epoxide, and the conformation of **1** was performed an X-ray diffraction experiment (Figure 3). Thus, it was concluded that the acetoxyl and *n*-octanoate groups are located at C-2 and C-4, respectively, and the epoxide is α-oriented.

The ^1H NMR spectrum of **2**,[6] $C_{28}H_{36}O_{14}$, was similar to that of **1**, except for the presence of two additional acetyl groups and the absence of an octanoate group. The chemical shift corresponding to H-3 (δ 6.19, br d, J=10.5 Hz) was shifted downfield by 1.21 ppm when compared to that of H-3 in **1**, suggesting that compound (**2**) was concluded to be a 3,4-diacetoxy-4-(deoctanoxy) derivative of **1**. This was confirmed by extensive NMR spectrum, including ^1H-^1H COSY, ^{13}C-^1H, and HMBC experiments (Figure 4). The relative stereochemistry of **2** was determined to be the same as in **1** by the proton-proton coupling constants and NOE experiments. The β-configurations of H-7, H-12, H-15, M-18, H-20, and H-12 followed from the NOEs from H-3 to H-7 and H-15, from H-7 to H-18, from H-9 to H-18 and H-20, and from H-12 to H-20. The α-orientations of H-2 and H-10 were deduced from the NOEs from H-2 to H-10 and H-16. Configurations of three acetyl groups at C-2, C-3, and C-4 were determined to be β from the coupling constants between H-2 and H-3 (J=0) and between H-3 and H-4 (J=10.3 Hz).

This is the first isolation of briarane diterpenes, in which the successive positions from C-2 to C-4 were oxidized, although there are a few briarane diterpenes possessing an acyl group at C-2 and an epoxide between C-3 and C-4.[7]

EXPERIMENTAL

General Experimental Procedures. Melting points were uncollected. UV and IR spectra were recorded on a UV-210 and a MASCO FT/IR 5300. NMR spectra were recorded with a 400 MHz JEOL NMR instruments using TMS as internal standard and $CDCl_3$ as solvents. MS were obtained with a JEOL XD-303. A Rigaku RAXIS-IV diifractometer was used in the X-Ray work.

Animal Material. Specimens of *Briareum* were collected at Bonotsu, Kagoshima prefecture. The reference sample (collection no. 222) was deposited at Department of Chemistry and Bioscience and identified by Mr. K. Takemura (Sankei Kagaku, Co., Ltd.).

Extraction and Isolation. The organisms (wet weight: 7.6 kg) was chopped into small pieces and extracted with MeOH (30 L) immediately after collection. The MeOH extract (22 g) was suspended in H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried over Na_2SO_4, filtered , and evaporated to dryness (9.6 g) . Portion (5 g) of the CH_2Cl_2 extract was absorbed on silica gel and subjected to chromatography on silica gel packed in hexane, fractions (100 mL) being collected as follows: 1-2 (CH_2Cl_2-hexane, 4:1), 3-34 (CH_2Cl_2), 5-6 (MeOH-CH_2Cl_2, 1:49), 7-8 (MeOH-CH_2Cl_2, 1:19), 9-10 (MeOH-CH_2Cl_2, 1:9), 11-12 (MeOH-CH_2Cl_2, 1:4), and 13-14 (MeOH). Fractions 6-7 (1.9 g), which showed Ichthyotoxic activity, were chromatographed on silica gel using MeOH and CH_2Cl_2 , increasing the proportion of MeOH to elute the fractions from the column. The fractions eluted with MeOH-CH_2Cl_2 (1:49) gave a residue (1.08 g) and crystalline prisms **1** (20 mg). The residue was again subjected to chromatography on silica gel with MeOH-CH_2Cl_2 (1.5:98.5) and then with MeOH-CH_2Cl_2 (1.6:98.4) afforded **2** (2.3 mg).

Compound (1). Prisms from EtOH, mp 183.8-184.1°C, $[\alpha]_D$ +33.0° (c 0.1, MeOH); UV (MeOH) λmax 206 (ε 5200); IR (KBr) νmax 3443, 1784, and 1741 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3H, t, J=7.0 Hz, H-28), 1.16 (3H, s, H-20), *ca.*. 1.29 (8H, overlapped, H-24, H-25, H-26, and H-27),

1.32 (3H, s, H-15), 1.63 (2H, m, H-23), 1.70 (3H, s, H-18), 2.08 (3H, d, J=1.1 Hz, H-16), 2.22 (3H, s, OAc), 2.27 (3H, s, OAc), 2.38 (3H, m, H-22 and OH), 2.58 (1H, d, J=3.8 Hz, H-10), 2.76 (1H, br s, OH), 3.69 (1H, t, J=6.2, H-12), 4.70 (1H, br s, H-2), 4.86 (1H, overlapped, H-4), 4.87 (1H, overlapped, H-3), 5.35 (1H, d, J=10.6 Hz, H-14), 5.50 (1H, br d, J=9.5 Hz, H-6), 5.72 (1H, d, J=9.5 Hz, H-7), 5.82 (1H, dd, J=6.2 and 10.4 Hz, H-13), and 5.92 (1H, d, J=3.8 Hz, H-9); (C_5D_5N) δ 0.79 (3H, t, J=7.0 Hz, H-28), 1.13 (8H, overlapped, H-24, H-25, H-26, and H-27), 1.48 (3H, s, H-20), 1.59 (2H, m, H-23), 1.73 (3H, s, H-15), 2.00 (3H, s, H-18), 2.05 (3H, s, OAc), 2.16 (3H, s, OAc), 2.29 (3H, br s, H-16), 2.33 (1H, m, H-22), 3.32 (1H, d, J=3.8 Hz, H-10 Hz), 3.68 (1H, br s, OH), 4.02 (1H, br d, J=6.3 Hz, H-12), 5.11 (1H, br s, H-2), *ca.*. 5.57 (2H, overlapped, H-3 and H-4), 5.80 (1H, br d, J=10.3 Hz, H-6), 5.83 (1H, d, J=10.3 Hz, H-14), 6.05 (1H, dd, J=6.3 and 10.3 Hz, H-13), 6.49 (1H, d, J=10.3 Hz, H-7), and 6.59 (1H, d, J=3.8 Hz, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.7 (q, C-18), 14.1 (q, C-28), 15.5 (q, C-15), 20.9 (q, MeCOO), 21.4 x 2 (q each, MeCOO and H-20), 22.6 (t, C-27), 24.9 (t, C-23), 25.5 (q, H-16), 28.9, (t, C-24 or C-25), 29.0 (t, C-25 or C-24), 31.6 (t, C-26), 34.3 (t, C-22), 43.8 (d, C-10), 47.1 (s, C-1), 64.5 (s, C-17), 65.4 (d, C-9), 70.2 (d, C-12), 71.3 (s, C-8), 71.5 (d, C-3), 73.6 (d, C-7), 73.7 (s, C-11), 76.6 (d, C-4), 76.7 (C-2), 124.2 (d, C-6), 125.1 (d, C-13), 137.9 (d, C-14), 141.0 (s, C-5), 168.4 (s, MeCOO), 169. 6 (s, MeCOO), 170.5 (s, C-19), and 173.5 (C-21); FABMS m/z 645.2904 $[M + Na]^+$ (calcd for $C_{32}H_{46}O_{12}Na$ 645.2987).

Compound (2). Amorphous, $[\alpha]_D$ +50.7° (c 0.07, MeOH); UV (MeOH) λmax 206 (ε 5800); IR (film) νmax 3505, 1786, and 1747 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.14 (3H, t, J=7.0 Hz, H-15), 1.15 (3H, s, H-20), 1.68 (3H, s, H-18), 2.01 (3H, s, MeCOO), 2.08 (3H, s, MeCOO), 2.15 (3H, br s, H-16), 2.17 and 2.30 (3H each, s, MeCOO), 2.69 (1H, br d, J=3.7 Hz, H-10), 2.74 (1H, br s, OH), 3.70 (1H, m, H-12), 4.70 (1H, br s, H-2), 5.12 (1H, br d, J=10.3 Hz, H-4), 5.48 (1H, d, J=10.3 Hz, H-14), 5.58 (1H, br d, J=9.9 Hz, H-6), 5.85 (1H, dd, J=6.6 and 10.3 Hz, H-13), 5.92 (1H, d, J=9.9 Hz, H-7), 5.96 (1H, br d, J=3.7 Hz, H-9), and 6.19 (1H, br d, J=10.3 Hz, H-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 10.0 (q, C-18), 15.5 (q, C-15), 20.6, 20.8, 20.9, and 21.2 (q, MeCOO x 4), 21.3 (q, C-20), 25.4 (q, C-16), 43.1 (d, C-10), 46.9 (s, C-1), 64.5 (s, C-9), 65.5 (s, C-17), 70.1 (d, C-12), 71.1 (d, C-3), 71.5 (s, C-8), 73.4 (d, C-7), 74.0 (s, C-11), 76.1 (s, C-4), 125.4 x 2 (d each, C-6 and C-12), 138.5 (d, C-14), 140.3 (s, C-5), 169.0, 170.0 x 2, and 170.1 (s each, MeCOO x 4), 170.7 (s, C-19); FABMS m/z 603.2048 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_{13}Na$ 603.2054).

Acetylation of 3. Treatment of **1** (4 mg) was acetylated with Ac_2O (0.5 mL) in pyridine (0.5 mL) to afford a tetraacetate **3** (4 mg); IR (film) νmax 3549, 1788, and 1748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J=7.3 Hz, H-15), 1.16 (3H, s, H-20), 1.23 (3H, br s, H-20), 1.27 (8H, m, H-24, H-25, H-26 and H-27), 1.56 (2H, overlapped, H-23), 1.67 (3H, s, H-18), 2.07 x 2 (3H each, s, MeCOO), 2.15 (3H each, s, MeCOO), 2.20 (3H, d, J=1.5 Hz, H-16), 2.26 (2H, m, H-22), 2.31 (3H each, s, MeCOO), 2.41 (1H, br s, OH), 2.77 (1H, d, H-10), 4.58 (1H, d, J=1.5 Hz, H-2), 4.69 (1H, d, J=6.3 Hz, H-12), 5.14 (1H, dd, J=0.7 and 10.3 Hz, H-4), 5.57 (1H, d, J=10.3 Hz, H-14), 5.59 (1H, br d, J=10.1 Hz, H-6), 5.92-5.97 (3H, overlapped, H-7, H-9, and H-13), [δ 5.94 (1H, d, J=9.8 Hz, H-7), δ 5.94 (1H, dd, 6.3 and 10.6 Hz), H-13 (δ 5.96, 1H, d, J=4.2 Hz, H-9) at 40°C], and 6.09 (1H, dd, J=1.5 and 10.3 Hz, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.8 (q, C-18), 14.1 (q, C-28), 15.5 (q. C-15), 20.5 and 20.9 x 2

(q each, s, MeCOO), 21.2 (q, s, C-19), 21.3 (q, s, MeCOO), 22.6 (t, C-27), 24.7 (t, C-23), 25.4 (q, C-15), 28.9 (t, C-25 or C-26), 29.0 (t, C-26 or C-25), 31.6 (t, C-26), 34.2 (t, C-22), 43.6 (d, C-10), 47.0 (s, C-1), 64.7 (s, C-17), 65.1 (d, C-9), 70.7 (d, C-3), 71.5 (s, C-8), 72.7 (s, C-11), 73.1 (d, C-12), 73.3 (d, C-7), 76.0 (d, C-4), 76.8 (d, C-2), 123.2 (d, C-13), 125.3 (d, C-6), 140.2 (d, C-14), 140.7 (s, C-5), 168.7, 169.7 x 2, 169.9 (each, s, MeCOO x 4), 170.5 (s, C-19), and 172.8 (s, C-21). (+) FABMS *m/z* 707 $[M + H]^+$ and (-) FABMS *m/z* 859 $[M + NBA]^-$.

X-Ray analysis of 1. Crystal data:$C_{32}H_{46}O_{12}\cdot C_2H_5OH$, colorless prisms, monoclinic space group C2(#5), a=21.240(4), b=19.15(1), c=9.073(2), b=95.01(1)°, V=3676Å^3, Z=4, Dx1.208 g/cm^3, F(000)=1440, μ(MoKα)=0.92 cm^{-1}, Intensity data were collected on a Rigaku RAXIS-IV diffractometer using graphite monochromated MoKα (λ=0.71070 Å) up to 2θ=55°. Of the total 3498 unique reflections, 2694 were observed [I>3σ (I)]. The structure was solved by direct methods (SIR92)[8] and expanded using Fourier techniques.[9] The non-hydrogen atoms were refined anisotropically. One mole of EtOH is contained in an asymmetric unit. Hydrogen atoms were included but not refined. It was refined by full-matrix least-squares and converged with R=0.056 and Rw=0.077. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at Rigaku Corporation.

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