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OLEANANE-TYPE TRITERPENES FROM *VIBURNUM AWABUKI*

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Key Word Index—*Viburnum awabuki*; Caprifoliaceae; wood; oleanane-type triterpene; erythrodiol; 1-oxo-erythrodiol; 11-oxo-erythrodiol; 13,28-epoxy-11-oleanene-3-one; castanopsone.

Abstract—Three new oleanane-type triterpenes have been isolated from the wood of *Viburnum awabuki*. Their structures have been elucidated as 3β,28-dihydroxy-12-oleanene-1-one, 3β,28-dihydroxy-12-oleanene-11-one and 13,28-epoxy-11-oleanene-3-one, respectively, by extensive analysis of spectroscopic data including comparison of ^{13}C NMR data with those of erythrodiol and some chemical transformations. The structure of 1-oxo-erythrodiol has been confirmed by X-ray crystallographic analysis.

INTRODUCTION

The leaves of *Viburnum awabuki* L. contain a number of vibsane-type diterpenes with novel eleven- and seven-membered carbon skeletons [1–3], whereas no diterpenes have been so far found in its wood and fruit [4]. As a part of our phytochemical studies on *V. awabuki*, we have continued to examine the chemical components in its wood, and now report on the isolation and characterization of three new oleanane-type triterpenes (**1**, **2**, and **4**) along with known castanopsone (**3**) [5] from this source.

RESULTS AND DISCUSSION

The wood of *V. awabuki* was extracted with methanol and the methanol extract partitioned between ethyl acetate and water. The ethyl acetate-soluble portion was fractionated by repeated CC on silica gel and Sephadex LH-20 to give three new oleanane-type triterpenes named 1-oxo-erythrodiol (**1**), 11-oxo-erythrodiol (**2**) and 13,28-epoxy-11-oleanene-3-one (**4**).

Compound **1** was assigned the molecular formula $C_{30}H_{48}O_3$, (HR-EI MS, *m/z* 456.3606 $[M]^+$). Its IR spectrum showed the presence of a hydroxyl group at 3393 cm^{-1} and a carbonyl group at 1698 cm^{-1}. Acetylation of **1** with Ac_2O–pyridine yielded the diacetate **1a** proving the presence of two hydroxy groups in the molecule of **1**. The ^1H NMR spectrum of **1** revealed the presence of seven tertiary methyl groups [δ_H 0.87, 0.89, 0.99, 1.02, 1.05, 1.19, and 1.30 (each 3H, *s*)], an isolated oxymethylene group [δ_H 3.21 and 3.55 (each 1H, *d*, J = 11.0 Hz)] as well as of an oxygen-bearing methine [δ_H 3.49 (1H, *dd*, J = 12.2, 4.9 Hz]. Additionally, the EI mass spectrum showed a typical fragment ion peak at *m/z* 234 due to a retro-Diels Alder cleavage on the C ring of a 12-oleanene-type triterpene. This spectral feature indicates that compound **1** is an oleanane-type triterpene having a ketone function. In fact, the ^{13}C NMR data (Table 1) of **1** were very similar to those of erythrodiol (**5**) [5] except for the presence of the carbonyl resonance at δ_C 212.4. The sole carbonyl group was placed at the C-1 position based on the HMBC correlations (Fig. 1.). Thus, 1-oxo-erythrodiol (**1**) was elucidated to be 3β,28-dihydroxy-12-oleanene-1-one, which has not been reported before. Finally, the proposed structure for **1** was confirmed by X-ray crystallographic analysis of the diacetate **1a** (Fig. 2).

Compound **2** was found to have the same molecular formula ($C_{30}H_{48}O_3$) (HR-EIMS, *m/z* 456.3601 $[M]^+$) and its ^1H NMR spectrum resembled that of **1** except for the H-9 and H-12 olefinic proton signals which appeared as singlets at δ_H 2.34 and 5.57, respectively. The presence of two hydroxy groups was confirmed by converting **2** into the diacetate **2a**. The spectral data [245 nm; 1651 cm^{-1}; δ_H 5.75 (1H, *s*, H-12); δ_C 200.1 (C-11)] of **2** suggested that a sole carbonyl group was involved in a conjugated system. In the HMBC experiment (Fig. 1), the H-9 singlet signal showed a long-range correlation with the carbonyl signal. This

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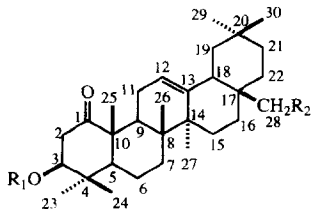
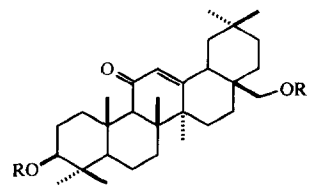
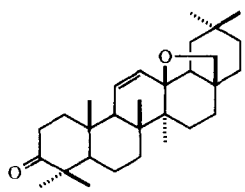


1 R_1 = H, R_2 = OH
1a R_1 = Ac, R_2 = OAc
3 R_1 = R_2 = H



2 R = H
2a R = Ac



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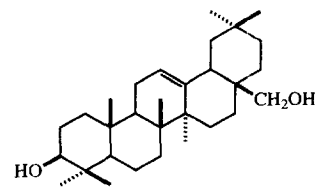

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Table 1. ^{13}C NMR data of compounds **1**–**5** (at 100 MHz, δ in $CDCl_3$)

	1	2	3	4	5
1	212.4	39.2	211.7	39.0	38.6
2	44.1	27.3	45.8	34.0	27.2
3	78.6	78.8	78.0	217.4	79.0
4	39.3	39.2	38.7	47.7	38.8
5	54.0	55.0	53.4	54.7	55.2
6	17.8	17.5	17.2	18.9	18.4
7	32.5	32.7	32.0	30.7	32.6
8	42.0	45.4	41.3	41.5	39.8
9	39.1	61.8	38.5	52.6	47.6
10	52.3	37.0	51.7	36.2	36.9
11	25.3	200.1	25.2	131.6	23.6
12	123.0	128.3	121.1	131.4	122.3
13	143.2	169.4	143.5	84.7	144.2
14	39.7	43.4	39.1	43.8	41.7
15	25.5	25.8	25.5	25.3	25.6
16	22.0	21.6	23.1	25.6	22.0
17	37.0	37.1	36.5	41.5	36.9
18	42.5	42.7	43.5	51.1	42.3
19	46.1	44.9	46.8	37.1	46.5
20	30.9	31.1	27.9	31.7	31.0
21	34.1	32.9	34.2	34.9	34.1
22	31.0	30.1	30.5	30.8	31.0
23	16.0	15.6	15.4	26.1	15.5
24	28.5	28.1	27.8	20.8	28.1
25	15.0	16.4	14.4	17.2	15.5
26	17.5	18.6	17.0	19.0	16.7
27	25.7	23.4	26.3	19.3	25.9
28	69.8	69.7	23.1	77.3	69.7
29	33.2	32.9	32.7	33.6	33.2
30	23.6	23.4	24.7	23.6	23.6

meant that the ketone function was located on the C-11 position in a 12-oleanene skeleton. Thus, 11-oxo-erythrodiol (**2**) represents as 3β,28-dihydroxy-12-oleanene-11-one, which is the first isolation of this aglycone although its glycosides are known [6].

Compound **4** was found to have the molecular formula $C_{30}H_{46}O_2$, (HR-EI MS, *m/z* 438.3504 $[M]^+$), and its IR spectrum showed the presence of a carbonyl group, (1703 cm^{-1}) which was also suggested by the ^{13}C NMR data at δ_C 217.4, but displayed no absorptions due to hydroxy groups. The NMR spectra (Table 1) of **4** disclosed the presence of seven tertiary methyl groups [δ_H 0.88, 0.95, 0.96, 1.03, 1.04, 1.09 and 1.13 (each 3H, *s*)], a disubstituted double bond [δ_H 5.42 (1H, *dd*, *J* = 10.4, 3.2 Hz, H-12) and 5.84 (1H, *dd*, *J* = 10.4, 1.4 Hz, H-11); δ_C 131.4 and 131.6] as well as of an isolated oxymethylene [δ_H 3.27 and 3.72; δ_C 77.3]. The sole carbonyl signal had distinct HMBC correlations with H_3-23 (δ_H 1.09) and H_3-24 (δ_H 1.04). These spectral data indicated that **4** was 3-oxo-oleanane-type triterpene. In the HMBC experiment (Fig. 1), the oxymethylene signals showed cross peaks to the oxygen-bearing quaternary carbon signal at δ_C 84.7, which in turn correlated to the olefinic proton signal at δ_H 5.42. The other olefinic signal at δ_H 5.84 coupled to the vicinal H-9. Thus, the disubstituted double bond had to be placed at the $\Delta^{11,12}$ position on the C-ring of the oleanane framework, and to account for the 8 degrees of unsaturation, the 6-membered ring had to be formed via an ether linkage between C-28 and C-13. Finally, **4** was shown by NOE to adopt the relative stereochemistry as shown in Fig. 3 Thus, the structure of compound **4** was assigned as 13,28-epoxy-11-oleanene-3-one.

In the leaves of *V. awabuki*, vibsane-type diterpenes, lupane-type triterpenes, coumarin glucosides [7], and flavonoid glycosides [8] are present, but no oleanane-type triterpenoids of the type reported herein have been found. It should be noted that a number of rearranged dammarane triterpenes have been isolated from the leaves of *Viburnum dilatatum* [9, 10]. On the

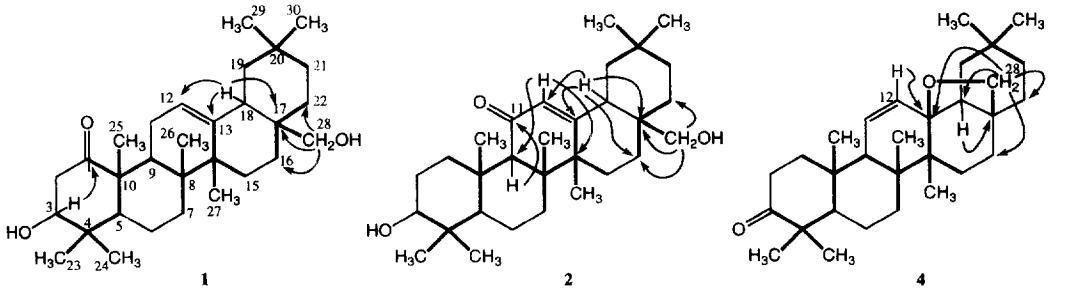

Fig. 1. Partial structures (bold lines) obtained by HMBC correlations from methyl proton signals and representative HMBC correlations (arrows) from particular proton signals of compounds **1**, **2** and **4**.

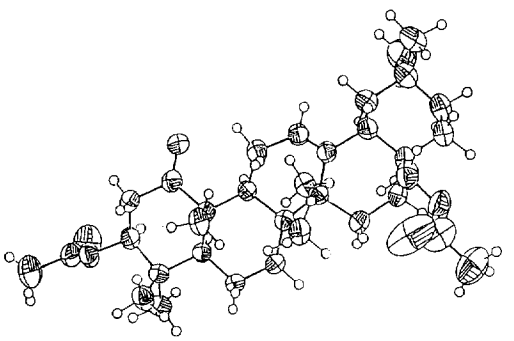

Fig. 2. ORTEP drawing of 1-oxo-erythrodiol diacetate (**1a**).

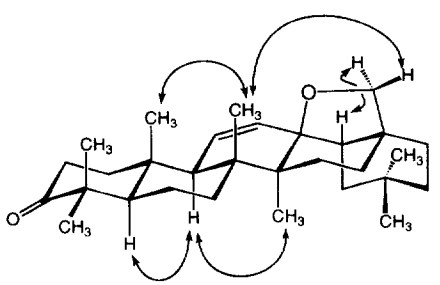

Fig. 3. Relative stereochemistry of **4** based on NOEs indicated by arrows.

other hand, from the wood and the fruits of *V. awabuki* benzofuran-type lignans [4], guaiane-type sesquiterpenoids [11], and oleanane-type and lupane-type triterpenoids have been isolated, but no vibsane-type diterpenoid characteristic of the chemical components of *V. awabuki* has been detected.

EXPERIMENTAL

^{1}H and ^{13}C NMR: TMS as int. standard; CC: silica gel (Merck, 230 ~ 400 mesh and Wakogel C-300) and Sephadex LH-20 (Pharmacia); TLC: precoated silica gel F254 (Merck). Spots were visualized by UV (254 nm) and 10% $CeSO_4$-H_2SO_4.

Plant material

V. awabuki was collected in Tokushima, Japan. A voucher specimen has been deposited in this institute.

Extraction and isolation

The MeOH extract was partitioned between EtOAc and water. The EtOAc-soluble portion (50 g) was sepd by CC on silica gel (Merck) alternately with *n*-hexane, *n*-hexane-EtOAc (9:1; 7:3; 4:6), EtOAc and EtOAc–MeOH (9:1) to give 6 frs (1–6). Fr. 4 (6.5 g) was again sepd by CC on silica gel (Merck) with CH_2Cl_2–EtOAc (1:1) to give 4 frs (7–10). Fr. 7 (1.5 g) was purified by repeated CC on silica gel (C-300) with CH_2–EtOAc (3:1) followed by CC on LH-20 with MeOH to give compounds **1** (18 mg) and **2** (6.2 mg). Fr. 3 (8.6 g) was sepd by CC on silica gel (C-300) with $CH_2Cl_2Cl_2$–EtOAc (10:1) to give 7 frs (11–17). Fr 16 (332 mg) was filtered to remove the precipitate. The filtrate was again sepd by CC on silica gel with CH_2Cl_2–EtOAc (4:1) to give 7 frs (18–24). Fr. 22 (49 mg) was purified by recycling HPLC [recycled ×6; JAIGEL-1H (20 × 600 mm i.d.), $CHCl_3$ (3.5 ml min^{-1})] followed by CC on LH-20 with $CHCl_3$–*n*-hexane (3:2) to afford castanopsone **3** (9 mg) and **4** (5 mg).

1-*Oxo-erythrodiol* (**1**)

Prisms, mp 218–221°, $[\alpha]_D^{24}$ +84 (*c* 1.55, $CHCl_3$). EIMS *m/z* (rel. int.): 456.3606 $[M]^+$ (calc. 456.3603 for $C_{30}H_{48}O_3$) (12), 426 $[M-30]^+$ (25), 234 $[M-C_{14}H_{22}O_2]^+$ (10); IR ν_{max}^{FT} cm^{-1}: 3393 (OH), 1698 (C=O). ^{1}H (400 MHz, $CDCl_3$): δ 0.87 (3H, *s*, H_3-30), 0.89 (3H, *s*, H_3-29), 0.90 (1H, *dd*, *J* = 12.7, 2.0 Hz, H-5), 0.99 (3H, *s*, H_3-26), 1.02 (3H, *s*, H_3-23), 1.05 (3H, *s*, H_3-24), 1.06 (1H, *m*, H-15), 1.13 (1H, *dd*, *J* = 13.2, 3.9 Hz, H-19), 1.19 (3H, *s*, H_3-27), 1.30 (3H, *s*, H_3-25), 1.44 (1H, *ddd*, *J* = 12.5, 12.5, 3.7 Hz, H-7), 1.50 (1H, *ddd*, *J* = 13.9, 13.9, 4.6 Hz, H-16), 1.73 (1H, *dd*, *J* = 13.4, 13.2 Hz, H-19), 1.83 (1H, *ddd*, *J* = 18.1, 6.8, 2.9 Hz, H-11), 1.89 (1H, *ddd*, *J* = 13.9, 13.9, 4.4 Hz, H-22), 1.98 (1H, *dd*, *J* = 13.2, 3.9 Hz, H-18), 2.23 (1H, *dd*, *J* = 6.8, 4.6 Hz, H-9), 2.32 (1H, *ddd*, *J* = 18.1, 4.9,

4.6 Hz, H-11), 2.37 (1H, *dd*, J = 12.0, 4.9 Hz, H-2), 3.03 (1H, *dd*, J = 12.2, 12.0 Hz, H-2), 3.21 (1H, *d*, J = 11.0 Hz, H-28), 3.49 (1H, *dd*, J = 12.2, 4.9 Hz, H-3), 3.55 (1H, *d*, J = 11.0 Hz, H-28), 5.20 (1H, *dd*, J = 4.9, 2.9 Hz, H-12); ^{13}C NMR: Table 1.

Acetylation of **1**

A mixture of **2** (5 mg), Ac_2O (0.4 ml) and pyridine (0.6 ml) was left to stand at room temp overnight. The reaction mixt was evapd *in vacuo*. The residue was chromatographed on silica gel [CH_2Cl_2–EtOAc (10:1)] to yield the diacetate **1a** (5.5 mg) as crystals, mp 195–195° (from MeOH). HR-EIMS *m/z* (rel. int.): 540.3807 $[M]^+$ (8) (calc. 540.3815 for $C_{34}H_{52}O_5$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (3H, *s*, H_3-30), 0.89 (3H, *s*, H_3-29), 0.96 (3H, *s*, H_3-26), 1.00 (1H, *s*, H_3-23), 1.05 (1H, *s*, H_3-24), 1.17 (3H, *s*, H_3-27), 1.30 (3H, *s*, H_3-25), 2.05 (6H, *s*, OAc), 2.47 (1H, *dd*, J = 12.4, 4.6 Hz, H-2α), 2.93 (1H, *dd*, J = 12.4, 12.0 Hz, H-2β), 3.68 (1H, *d*, J = 11.0 Hz, H-28), 4.00 (1H, *d*, J = 11.0 Hz, H-28), 4.75 (1H, *dd*, J = 12.0, 4.6 Hz, H-3), 5.21 (1H, *t*, J = 3.5 Hz, H-12).

11-*Oxo-erythrodiol* (**2**)

Prisms, mp 145–147°, $[\alpha]_D^{24}$ +61 (*c* 0.77, $CHCl_3$). UV λ_{max}^{EtOH} nm: 245 (ε 7600); EIMS *m/z* (rel. int.): 456.3601 $[M]^+$ (calc. 456.3603 for $C_{30}H_{48}O_3$), 441 $[M-15]^+$ (71), 248 $[M-C_{14}H_{24}O]^+$ (100). IR ν_{max}^{FT} cm^{-1}: 3406 (OH), 1651 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (1H, *dd*, J = 11.5, 2.5 Hz, H-5), 0.81 (3H, *s*, H_3-24), 0.89 (3H, *s*, H_3-29), 0.92 (3H, *s*, H_3-30), 0.98 (1H, *ddd*, J = 13.4, 13.4, 4.1 Hz, H-1), 1.00 (3H, *s*, H_3-23), 1.11 (3H, *s*, H_3-26), 1.13 (3H, *s*, H_3-25), 1.15 (1H, *dd*, J = 13.4, 4.2 Hz, H-19), 1.18 (1H, *ddd*, J = 13.9, 4.4, 2.2 Hz, H-15), 1.33 (1H, *dd*, J = 13.9, 3.9 Hz, H-21), 1.35 (1H, *ddd*, J = 13.9, 4.4, 4.4 Hz, H-16), 1.39 (3H, *s*, H_3-27), 1.65 (2H, *m*, H-2), 1.73 (1H, *dd*, J = 13.9, 13.4 Hz, H-19), 1.77 (1H, *ddd*, J = 13.9, 13.9, 4.4 Hz, H-15), 2.15 (1H, *dd*, J = 13.9, 4.2 Hz, H-18), 2.34 (1H, *s*, H-9), 2.78 (1H, *ddd*, J = 13.4, 3.7, 3.7 Hz, H-1), 3.22 (1H, *d*, J = 11.0 Hz, H-28), 3.23 (1H, *dd*, J = 10.5, 5.5 Hz, H-3), 3.47 (1H, *d*, J = 11.0 Hz, H-28), 5.57 (1H, *s*, H-12); ^{13}C NMR: Table 1.

Acetylation of **2**

A mixt of **2** (3 mg), Ac_2O (0.2 ml) and pyridine (0.5 ml) was left overnight. The reaction mixt was evapd *in vacuo*. The residue was chromatographed on silica gel [CH_2Cl_2–EtOAc (3:1)] to yield the diacetate **2a** (2.5 mg) as an amorphous powder. EIMS *m/z* (rel. int.): 540.3818 $[M]^+$ (26) (calc. 540.3815 for $C_{34}H_{52}O_5$), 481 $[M-OAc]^+$ (25), 271 (100); IR ν_{max}^{FT} cm^{-1}: 1738 (C=O), 1660 (conj. C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79 (3H, *s*), 0.88 (6H, *s*), 0.90 (3H, *s*), 0.92 (3H, *s*), 1.04 (1H, *dddd*, J = 13.7, 13.7, 3.7, 3.7 Hz, H-1α), 1.12 (3H, *s*), 1.15 (3H, *s*), 1.38 (3H, *s*), 2.01 (1H, *ddd*, J = 13.7, 13.7, 4.4 Hz, H-16), 2.05 (3H, *s*, OAc), 2.06 (3H, *s*, OAc), 3.69 (1H, *d*, J = 11.2 Hz, H-28), 3.95 (1H, *d*, J = 11.2 Hz, H-28), 4.51 (1H, *dd*, J = 11.7, 4.9 Hz, H-3), 5.57 (1H, *s*, H-12).

13,28-*Epoxy*-11-*oleanene*-3-*one* (**4**)

Prisms, mp 200–203°, $[\alpha]_D^{24}$ +106 (*c* 0.20, $CHCl_3$). EIMS *m/z* (rel. int.): 438.3504 $[M]^+$ (22) (calc. 438.3498 for $C_{30}H_{46}O_2$), 420 $[M-18]^+$ (100), 407 $[M-OMe]^+$ (59), 392 $[M-C_2H_6O]^+$ (13); IR ν_{max}^{FT} cm^{-1}: 1703 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, *s*, H_3-30), 0.95 (3H, *s*, H_3-27), 0.96 (3H, *s*, H_3-29), 1.03 (3H, *s*, H_3-25), 1.04 (3H, *s*, H_3-24), 1.09 (3H, *s*, H_3-23), 1.11 (1H, *ddd*, J = 13.2, 12.9, 6.0 Hz, H-16), 1.13 (1H, *s*, H_3-26), 1.21 (1H, *ddd*, J = 13.6, 4.1, 2.5 Hz, H-21), 1.27 (1H, *m*, H-19), 1.31 (1H, *dd*, J = 12.6, 3.0 Hz, H-5), 1.35 (1H, *dd*, J = 13.6, 4.4 Hz, H-21), 1.39 (1H, *m*, H-1), 1.43 (1H, *m*, H-22), 1.49 (2H, *m*, H-6), 1.52 (1H, *m*, H-22), 1.66 (1H, *m*, H-18), 1.73 (1H, *d*, J = 12.6 Hz, H-19), 1.80 (1H, *ddd*, J = 13.2, 13.2, 5.8 Hz, H-15), 1.93 (1H, *m*, H-9), 2.01 (1H, *m*, H-16), 2.07 (1H, *ddd*, J = 13.2, 7.3, 3.8 Hz, H-1), 2.40 (1H, *ddd*, J = 16.0, 6.9, 3.8 Hz, H-2), 2.60 (1H, *ddd*, J = 16.0, 11.0, 7.3 Hz, H-2), 3.27 (1H, *dd*, J = 6.9, 2.1 Hz, H-28), 3.72 (1H, *d*, J = 6.9 Hz, H-28), 5.42 (1H, *dd*, J = 10.4, 3.2 Hz, H-12), 5.84 (1H, *dd*, J = 10.4, 1.4 Hz, H-11); ^{13}C NMR: Table 1.

X-ray crystallographic analysis of **1a**

X-ray analysis was performed on a Mac Sciences MXC 18 diffractometer with Cu Kα (λ = 1.54178) radiation. The structure of **1a** was solved by the direct method using *CRYSTAN SIR* 92 and refined by full-matrix least-squares using *CRYSTAN*. Crystal data: $C_{34}H_{52}O_5$: monoclinic; space group P21 (#4); a = 18.551 (4), b = 12.212 (4), c = 6.974 (2) Å, β = 94.49 (2)°; Dx = 1.14 g cm^{-3}; z = 2; μ(Cu Kα) = 5.13 cm^{-1}; final R = 0.050.

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