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SESQUITERPENE AND OTHER CONSTITUENTS OF THE LIVERWORT *DUMORTIERA HIRSUTA*

MASAO TOYOTA, TATSUHIKO YOSHIDA, JUNKO MATSUNAMI and YOSHINORI ASAKAWA

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

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Key Word Index—*Dumortiera hirsuta*; Wiesnerellaceae Grolle; Marchantiales; bisabolane and germacrane-type sesquiterpenes; bis-bibenzyl derivatives.

Abstract—A new sesquiterpene and 13 known compounds were isolated from the ether extract of *Dumortiera hirsuta*. Their structures were established by spectral and chemical evidence and/or X-ray crystallographic analysis. The structure of the new compound was shown to be (4*S*, 6*R*)-2,7,10-bisbolatrien-4-ol. The absolute configuration of (4*S*, 7*R*)-germacra-1(10)*E*, 5*E*-dien-11-ol was established by chemical degradation, although it has been reported previously on the basis of the comparison of the magnitude of optical rotation with that of a related known compound. The tentative structure of isomarchantin C has been reported previously, but it has now been established by X-ray crystallographic analysis. Copyright © 1996 Elsevier Science Ltd

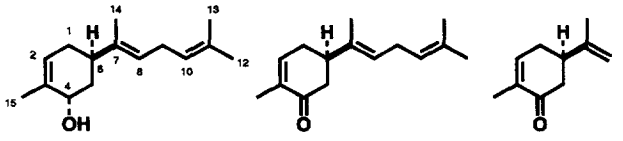
INTRODUCTION

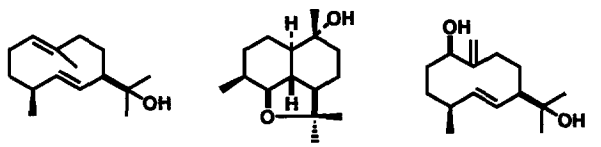
Our phytochemical studies of bryophytes have been performed from the chemosystematic point of view [1]. The Hepaticae occasionally produce their own peculiar bisbibenzyl derivatives. These have not been found in higher plants, fungi or marine organisms. The marchantin series has been isolated from *Marchantia* species (Marchantiales) belonging to the thallic liverworts. The thallic liverwort *Dumortiera hirsuta* grows on wet rocks and is known in Japan. A previous phytochemical study of *Dumortiera hirsuta* led to the isolation of 4,5-dehydronerolidol [1] and to the detection of ten sesquiterpene hydrocarbons by GC-mass spectrometry [2]. In our previous work, we reported the absence of bis-bibenzyl derivatives in this species [3]. Further fractionation of the ether extract of *D. hirsuta* resulting in the isolation of cyclic bis-bibenzyl derivatives. Here, we wish to report the isolation of a new bisabolane-type sesquiterpene alcohol, in addition to 13 known compounds including three bis-bibenzyl derivatives, having the marchantin or riccardin type linkage.

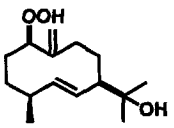
RESULTS AND DISCUSSION

The ether extract of *Dumortiera hirsuta* was repeatedly chromatographed on silica gel, Sephadex LH 20 and further purified by HPLC on a normal phase column to give a new sesquiterpenoid (4*S*, 6*R*)-2,7,10-bisabolatrien-4-ol (**1**), (4*S*, 7*R*)-germacra-1 (10)*E*,5*E*-dien-11-ol (**4**), (4*S**, 5*S**, 6*R**, 7*R**)-1(10)*E*-lepidozen-5-ol (**11**), marchantin C (**13**), isomarchantin C (**14a**), riccardin C (**15**) and lunularin (**16**).

The EI-mass spectrum of **1** gave a molecular ion peak at *m/z* 220 and its HR-mass spectrum indicated a







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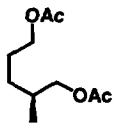

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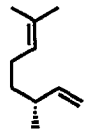

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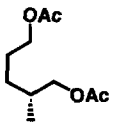

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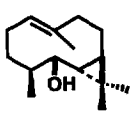

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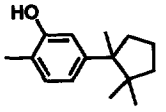

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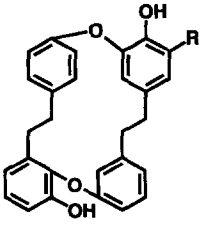
molecular formula $C_{15}H_{24}O$, confirming four degrees of unsaturation. The IR spectrum of **1** exhibited the presence of a hydroxyl group at 3354 cm^{-1}. The ^1H NMR and ^{13}C NMR (Tables 1 and 2) spectra of **1** not only provided evidence for the presence of a secondary hydroxyl group, but also displayed signals for four vinyl methyl groups and three trisubstituted double bonds. The three double bonds accounted for three of the four units of unsaturation and, therefore, **1** was a monocyclic compound.

Analysis of the HMBC spectra (summarized in Table 3) supported the structural assignment. In particular, the long range $^1H-^{13}C$ correlation of H-12 with C-13, C-11 and C-10, supported the positioning of the two vinyl methyl groups at C-11. Further correlations exhibited the presencae of a 1,5-dimethyl-1,4-hexadienyl moiety of **1**.

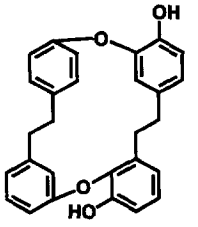
There is only one possible secondary hydroxyl position which is at C-4, since a derived ketone showed an intense α, β-unsaturated carbonyl absorption at 1676 cm^{-1} in the IR spectrum of **2**. Support for this assignment was obtained by irradiation of H-4 which caused a triplet ($J = 14$ Hz) of an axial proton H-5 [δ_H 1.49 (*ddd*, $J = 14$, 14, 4 Hz)] which was in turn coupled to the equatorial H-5 and H-6 protons in the ^1H NMR of **1**. This also indicated that the secondary hydroxyl group had an axial configuration. Consideration of these chemical and spectral data led to the conclusion that the planar structure of **1** was 2,7,10-bisabolatrien-4-ol. The relative stereochemistry of **1** was established by difference NOE experiments of **1** and **2** (Table 1).

The absolute configuration of **1** was established by comparing the CD spectral data of **2** with those of *d* and *l*-carvone. Since a positive absorption band due to $\pi-\pi^*$ transition at 244 nm in the CD spectrum of **2** was identical to that of *l*-carvone (**3**), it was clear that the absolute configuration of **2** was (6*R*)-2,7,10-bisabolatrien-4-one. Accordingly, the absolute configuration of **1** was established to be (4*S*, 6*R*)-2,7,10-bisabolatrien-4-ol.

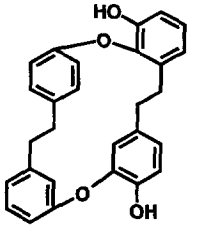
The EI-mass spectrum of **4** gave $[M]^+$ at *m/z* 222. Absorption at 3370 cm^{-1} showed the presence of a hydroxyl group. However, no carbonyl group was observed in the IR spectrum of **4**. Since the ^{13}C NMR spectrum of **4** showed the presence of oxygenated quaternary carbon at δ 71.8 and no carbinyl proton was found in its ^1H NMR spectrum, it was clear that the hydroxyl group was tertiary. Analysis of the HMQC and HMBC spectra supported the planar structure of **4**. The above data were confirmed by an X-ray crystallographic analysis on crystals of **4** obtained from *n*-hexane solution. The stereoscopic view of the crystal of **4** is shown in Fig. 1. All the features mentioned above



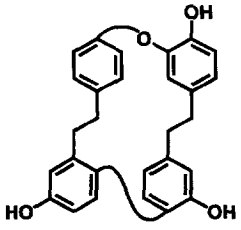
13 R=H
17 R=OH



14a



14b



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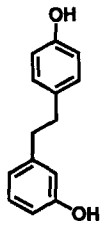

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Table 1. ^{1}H NMR data for compounds **1** and **2**

H	1	NOE observed ^{1}H	2	NOE observed ^{1}H
1	1.85 (2H, *m*)		1.69 (*m*) eq 1.87 (*m*) ax	
2	5.40 (*brs*)		6.10 (*brd*, 6)	
3				
4	3.74 (*brs*)			
5	1.49 (*ddd*, 14, 14, 4) ax 1.59 (*brd*, 14) eq		2.13 (*dd*, 16, 14) ax 2.35 (*ddd*, 16, 4, 2) eq	
6	3.05 (*m*)	H-8	3.10 (*m*)	H-8
7				
8	5.28 (*brt*, 7)		5.14 (*brd*, 7)	
9	2.86 (2H, *brt*, 7)		2.60 (2H, *brt*, 7)	
10	5.26 (*brt*, 7)		5.10 (*brt*, 7)	
11				
12	1.66 (*s*)	H-10	1.63 (*d*, 1)	H-10
13	1.58 (*s*)		1.49 (*s*)	
14	1.59 (*s*)		1.40 (*d*, 1)	H-5 ax.
15	1.72 (*d*, 2)	H-2, 4	1.83 (*s*)	

Assignments were carried out by HMBC spectrum.
Measured in C_6D_6.

Table 2. ^{13}C NMR data for compound **1**

C	1
1	30.1
2	125.1
3	134.8
4	68.2
5	36.9
6	29.6
7	137.9
8	124.1
9	26.9
10	125.2
11	131.7
12	25.9
13	17.7
14	19.7
15	21.3

Measured in C_6D_6.

Table 3. ^{1}H–^{13}C long range correlations of **1**

^{1}H	^{13}C
2	1, 4, 6, 15
4	2, 3, 6, 15
5	6
6	1, 2, 5, 7, 14
8	6, 9, 14
9	7, 8, 10, 11
10	9, 12, 13
12	10, 11, 13
13	11, 12
14	6
15	2, 3, 4

Observed by HMBC spectrum.

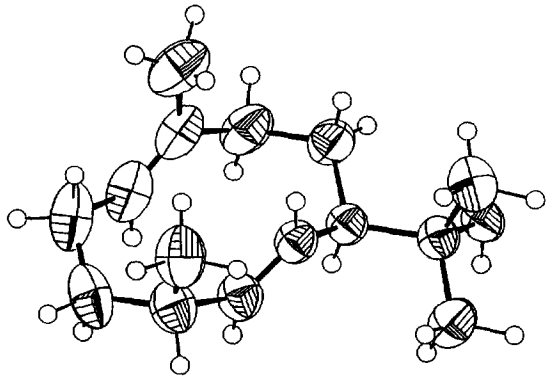


Fig. 1. A computer generated drawing of the final X-ray model of **4**.

show that the structure and relative configuration of **4** are established as shown.

Although compound **4** was isolated previously from *Streptomyces citreus* CBS 109.60 and the structure established, its absolute configuration was ambiguously assigned by comparing the magnitude of its optical rotation with that of the known compound (4*S*, 7*R*)-1(10)*E*, 5*E*-germacradiene [4]. Furthermore, the magnitude of optical rotation of **4** {$[\alpha]_D$ −153.3° ($CHCl_3$, *c* 1.5)} did not correspond to the value {$[\alpha]_D$ −82° ($CHCl_3$, *c* 0.23)} in the literature [4]. For this reason, an attempt was made to establish the absolute configuration of **4**. Ozonolysis of **4** yielded a mixture of aldehydes which were acetylated without purification. This was followed by reduction with sodium borohydride to give an acetate **8** ($[\alpha]_D$ −2.0°). The same degradation of (−)-β-citronellene (**9**) afforded an acetate **10** ($[\alpha]_D$ +2.6°) whose spectral data were identical to those of acetate **8**, except for the optical rotation. Thus, the absolute stereochemistry at C-4 had the *S*-configuration. Accordingly, the structure of **4** was

unambiguously determined as (4*S*, 7*R*)-germacra-1(10)*E*, 5*E*-dien-11-ol whose absolute configuration was identical to that previously reported [4].

On the other hand, the spectral data of **4** did not agree with those of allohedycariol, isolated from *Ferula communis* and its planar structure has been determined as 1(10)*E*, 5*E*-germacra-dien-11-ol [5]. This indicates that allohedycariol is a steroisomer of **4**.

From the methanolic extract of the Indian *Marchantia polymorpha* and *M. palmata*, a cyclic bisbibenzyl-type compound isomarchantin C [6] has been isolated. Its structure has been most favourably assigned to be **14a** by the spectral data and the co-occurrence of the compound with marchantin A (**17**) [7, 8]. However, the extensive NMR analysis of isomarchantin C cannot fully characterize the structure, and the structure of **14b** is not excluded for isomarchantin C. During the course of the fractonation of polar constituents, three cyclic bis-bibenzyl-type compounds, isomarchantin C (**14a**), marchantin C (**13**) [9] and riccardin C (**15**) [10] were isolated, although we reported previously the absence of these derivatives in this species [3]. The structure of isomarchantin C was clarified by an X-ray crystallographic anlysis on crystals obtained from a mixture of *n*-hexane and ethyl acetate solution. The stereoscopic view of the crystal of isomarchantin C (**14a**) is shown in Fig. 2 and the structure of **14a** was reconfirmed as shown.

When compound **4** was allowed to stand at room temperature for a long time, it easily converted to **5**, **6**, and **7**. During the investigation on the constituents of *D. hirsuta*, compound **5** has been isolated from an old ether extract of the species. Therefore, it was suggested that **5** might be an artefact in this species. GC-mass spectral analysis of the ether extract of *D. hirsuta*, collected from various places, revealed the presence of chemotypes in Japan as shown in Table 4. Even when the same species of liverworts are analysed the constituents are often different. *Dumortiera hirsuta* is divided into at least three types phytochemically, one of which contains compound **4** and γ-cadinene (**18**), and the other two types, which contain β-elemene (**19**), elemol (**20**), and 3,4-dehydronerolidol (**21**) as a major constituent, respectively (Table 4).

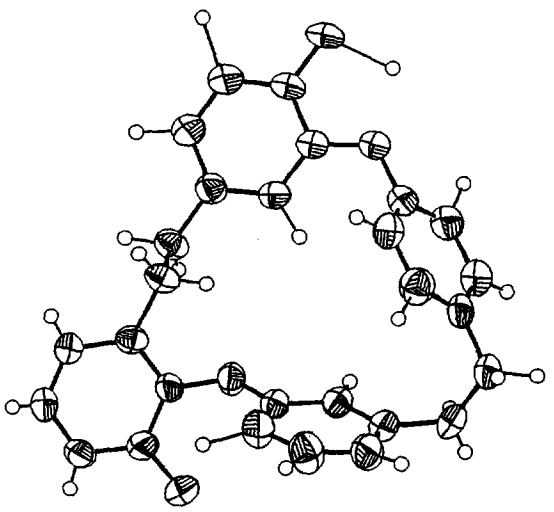
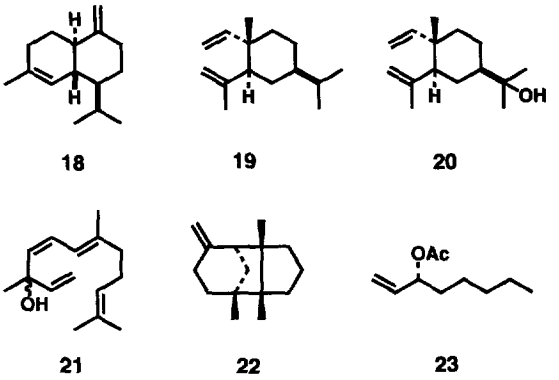


Fig. 2. A computer generated drawing of the final X-ray model of isomarchantin C (**14a**).



18 **19** **20**

21 **22** **23**

EXPERIMENTAL

General. TLC was carried out on silica gel precoated glass plates with *n*-hexane–EtOAc (1:1 and 4:1). Detection was with Godin reagent [11]. For normal phase column chromatography (CC), silica gel 60 (40–63 μm) was used. The mixt. of CH_2Cl_2–MeOH (1:1) was used for CC on Sephadex LH-20 as solvent.

Spectral data. NMR spectra were recorded at 100 or 50 MHz for ^{13}C and 400 or 200 MHz for ^{1}H. EI-MS were measured at 70 eV. The temperature programming of GC-MS analysis was performed from 50° isothermal for 3 min, then 50–250° at 5° min^{-1}, and finally isothermal at 250° for 15 min. The injection temp was 250°. A fused silica column coated with DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm) was used.

Plant material. Dumortiera hirsuta (Sw.) Nees (2.14 kg) was collected in August, 1995 at Hatta-cho (Altitude 250 m), Tokushima, Japan. A voucher specimen (#95158) is deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and isolation. The purified liverwort was dried for 3 days, ground mechanically and then extracted with Et_2O for 8 days and with MeOH for 2 months. The Et_2O extract (55.7 g) of *D. hirsuta* was chromatographed on silica gel using an *n*-hexane–EtOAc gradient, giving 8 frs (I–VIII). Fr. II (4.06 g) was rechromatographed on Sephadex LH-20 to give a mixt. containing 2-hydroxycuparene (**12**) whose GC-MS was identical to that of an authentic sample. Fr. IV (4.54 g) was rechromatographed on Sephadex LH-20 and repurified by HPLC on silica gel to give **1** (11.4 mg), **4** (420 mg), phytol (97.6 mg) and **11** (24.1 mg). The spectral data of **11** was identical to those of (4*S**, 5*S**, 6*R**, 7*R**)-1(10)*E*-lepidozen-5-ol [12]. Fr. VII (10.1 g) was rechromatographed on Sephadex LH-20 to given sitosterol, stigmasterol and a

Table 4. The distribution of terpenoids in three chemotypes of *D. hirsuta*

Liverwort	**4**	**18**	**19**	**20**	**21**	**22**	**23**	Collection locations
D. hirsuta (95158)	+++++++	+++++				+++		Hatta, Tokushima
(96001)	+++++−	+++++	+−			++	+	Tosashimizu, Kochi
(DH1017)	++++++++	++++				+++	−	Hatta, Tokushima
(DH3412)			++++++	++++++		++++	+	Tosa, Kochi
(96016)	+++		+++++	+++++++		++	−	Katsuura, Tokushima
(DH1224)			++++	+++++++		+++	−	Hatta, Tokushima
(DH3349)					+++++++ ++++	+	+	Kamiyama, Tokushima
(DH3422)					+++++++ +++	++	−	Yuki, Hiroshima
(DH1)					+++++++ +++++	+	+	Kamiyama, Tokushima

Symbols used: −, less than 1%; +, 1–5% contents.

mixt. containing isomarchantin C and marchantin C. The mixt. was further purified by recrystallization using *n*-hexane–EtOAc and afforded isomarchantin C (**14a**) (1.38 g; 2.5% of the total extract) and marchantin C (**13**) (461 mg; 0.8%).

The methanolic extract was partitioned between EtOAc and *n*-BuOH. The EtOAc extract (11.9 g) was repeatedly chromatographed on Sephadex LH-20 and purified by HPLC on silica gel yielded lunularin (**16**) (3.5 mg), 4-hydroxybenzaldehyde (3.4 mg) and a mixt. containing riccardin C (**15**). The mixt. (41.2 mg) in Me_2CO was methylated with CH_3I in the presence of excess dry K_2CO_3, then purified by CC on silica gel to give riccardin C trimethyl ether. The polar fraction (45.3 mg) of the first CC of the methanolic extract was chromatographed on Sephadex LH-20 and gave protocatechuic acid (3.7 mg) and luteolin (28.4 mg).

(4S, 6R)-2, 7, 10-*Bisabolatrien*-4-*ol* (**1**). $[\alpha]_D$ −9.7 ($CHCl_3$, *c* 1.5) FT-IR ν_{max}^{neat} cm^{-1}: 3354, 1446 and 1377. HR-MS: found 220.1833 $C_{15}H_{24}O$, requires 220.1829; EI-MS *m/z* (rel. int.): 220 $[M]^+$ (27), 202 (11), 164 (11), 149 (11), 146 (16), 136 (17), 135 (35), 131 (17), 121 (22), 119 (12), 110 (14), 109 (100), 108 (11), 107 (19), 105 (18), 95 (20), 94 (14), 93 (68), 92 (13), 91 (20), 84 (29), 82 (14), 81 (19), 80 (14), 79 (16), 77 (14), 69 (18), 67 (18).

Oxidation of **1** *with pyridinium dichromate (PDC).* To PDC (37.6 mg) in CH_2Cl_2 (2 ml) was added **1** (11.4 mg) in CH_2Cl_2 and then the mixt. was stirred for 6 hr at room temp. The resulting mixt. (8.3 mg) was filtered and purified by HPLC on silica gel to yield **2** (3.9 mg).

(6R)-2, 7, 10-*Bisabolatrien*-4-*one* (**2**). FT-IR ν_{max}^{neat} cm^{-1}: 1676, 1449, 1377, 1246 and 1107. EI-MS *m/z* (rel. int.): 218 $[M]^+$ (10), 203 (10), 175 (25), 162 (50), 147 (55), 136 (40), 121 (30), 119 (30), 109 (100), 107 (35), 93 (65), 91 (40), 82 (30), 79 (30), 77 (30), 67 (30). CD: $\Delta\varepsilon_{244\,nm}$ +3.17 (MeOH; *c* 3.3×10^{-4} M). UV λ_{max} (MeOH) nm (log ε): 235 (4.34).

(4S, 7R)-*Germacra*-1, (10)E, 5E-*dien*-11-*ol* (**4**). mp 72–73°, $[\alpha]_D$ −153.3 ($CHCl_3$, *c* 1.5) {lit. $[\alpha]_D$ −82 ($CHCl_3$, *c* 0.23) [4]}. The spectral data were identical to those reported in the literature [4].

Ozonolysis of (4S, 7R)-*germacra*-1, (10)E, 5E-*dien*-11-*ol* (**4**) *and* (−)-*β*-*citronellene* (**9**). Ozone was bubbled into the soln of **4** (103 mg) and **9** (165 mg) in CH_2Cl_2–MeOH (1:1, 10 ml) at −78° for 20 min. $NaBH_4$ (500 mg) was added to the mixt. which was then stirred for 23 hr at room temp. After acetylation of the resulting mixt. it afforded an acetate **8** (from **4**; 35.5 mg) and **10** (from **9**; 39.1 mg). **8**; $[\alpha]_D$ −2.0 ($CHCl_3$, *c* 3.6). FT-IR ν_{max} cm^{-1}: 1742, 1466, 1368, 1236 and 1038. EI-MS *m/z* (rel. int.): 142 $[M-AcOH]^+$ (1), 112 (6), 100 (6), 99 (6), 82 (48), 70 (10), 69 (15), 67 (32), 61 (10), 54 (9), 43 (100). CI-MS *m/z* (rel. int.): 203 $[M+H]^+$ (100), 143 (12), 83 (28). ^1H NMR (200 MHz; $CDCl_3$): δ 0.95 (3H, *d*, *J* = 6.8 Hz), 1.10–1.90 (5H, *m*), 2.05 and 2.06 (each 3H, *s*), 3.92 and 4.06 (each 2H, *m*). ^{13}C NMR (50 MHz; $CDCl_3$): δ 16.6, 20.9 (×2), 25.9. 29.5, 32.2, 64.5, 69.0, 171.0 (×2). **10**; $[\alpha]_D$ +2.6 ($CHCl_3$, *c* 4.7). The spectral data were identical to those of **8**.

Crystal data for (4S, 7R)-*germacra*-1, (10)E, 5E-*dien*-11-*ol* (**4**) *and isomarchantin C* (**14a**). Refraction data were measured with a Mac Science MXC18 diffractometer using copper radiation CuK_α (λ = 1.54178). **4**; Orthorhombic space group $P3_2$ (No. 145). *a* = 14.046 (2), *b* = 14.046 (2), *c* = 6.472 (2) Å; *V* = 1098.0(5) $Å^3$; *Z* = 3, D_c = 1.34 g cm^{-3}, μ_{Cu} = 3.95 cm^{-1}. Crystal size; 0.8 × 0.7 × 0.5 mm. Final residuals *R* and R_w were 0.040, 0.057. **13**; Orthorhombic space group $P2_12_12_1$ (No. 19). *a* = 11.233 (2), *b* = 25.151 (4), *c* = 9.957 (2) Å; *V* = 2813.2 (9) $Å^3$; *Z* = 4, D_c = 1.17 g cm^{-3}, μ_{Cu} = 5.52 cm^{-1}. Crystal size: 0.5 × 0.7 × 0.4 mm. Final residuals *R* and R_w with 0.069, 0.099.

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