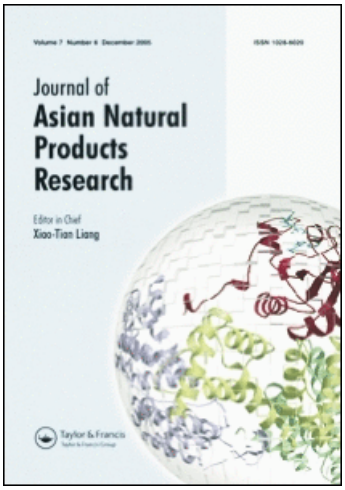

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Absolute configuration determination of angular dihydrocoumarins from *Peucedanum praeruptorum*

Hong-xiang Lou[a]; Long-ru Sun[a]; Wen-tao Yu[b]; Pei-hong Fan[a]; Lei Cui[a]; Yan-hui Gao[a]; Bin[a]; Dong-mei Ren[a]; Mei Ji[a]
[a] Department of Natural Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan, China [b] State Key Laboratory of Crystal Materials, School of Material Science and Engineering, Shandong University, Jinan, China

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ABSOLUTE CONFIGURATION DETERMINATION OF ANGULAR DIHYDROCOUMARINS FROM *PEUCEDANUM PRAERUPTORUM*

HONG-XIANG LOU[a,*], LONG-RU SUN[a], WEN-TAO YU[b], PEI-HONG FAN[a], LEI CUI[a], YAN-HUI GAO[a], BIN MA[a], DONG-MEI REN[a] and MEI JI[a]

[a]*Department of Natural Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China;* [b]*State Key Laboratory of Crystal Materials, School of Material Science and Engineering, Shandong University, Jinan 250100, China*

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From *Peucedanum praeruptorum,* one new khellactone ester (3′*R*)-*O*-acetyl-(4′*S*)-*O*-angeloylkhellactone (**3**), as well as four known angular dihydropyranocoumarins (**1**, **2**, **4**, **5**) have been isolated. On the basis of NMR spectra and X-ray crystallography, their structures were determined. We have elucidated their absolute configuration by either chiral separation of their alkaline hydrolysis products with Rp-18 HPLC eluted with 5% hydroxypropyl-β-cyclodextrin (β-HCD) or by measurement of their CD spectra. A general rule relating the position and absolute stereochemistry of the khellactone esters to the sign of their Cotton effects in CD curves is proposed.

Keywords: *Peucedanum praeruptorum*; Umbelliferae; Angular dihydropyranocoumarins

INTRODUCTION

Angular dihydropyranocoumarins occur in plant species of the genera *Peucedanum* [1], *Seseli* [2], *Musineon* [3], *Arracacia* [4] etc. These types of coumarins in *Peucedanum praeruptorum* have been extensively studied [1,5–7]. In investigating calcium antagonists from natural products [8] we isolated one new khellactone ester, (3′*R*)-*O*-acetyl-(4′*S*)-*O*-angeloylkhellactone (**3**), as well as four known angular dihydropyranocoumarins (**1**, **2**, **4** and **5**) from the light-petroleum-soluble fraction of the roots of *P. praeruptorum.* Here we reported their absolute configuration determination by a combination of spectral data (CD) and chemical conversion into the khellactone stereoisomers by alkali hydrolysis and subsequent analysis by Rp-18 HPLC with 5% hydroxypropyl-β- cyclodextrin (β-HCD) as mobile phase.

*Corresponding author. E-mail: louhongxiang@sdu.edu.cn

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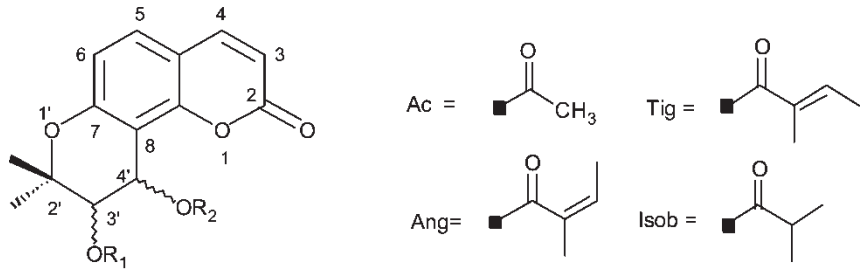

	3'	4'	R_1	R_1
1	┈	┈	Ang	Ac
2	┈	━	Ac	Ang
3	━	┈	Ac	Tig
4	━	━	Ac	Ang
5	┈	━	Ac	Isob
6	┈	┈	H	H
7	┈	━	H	H
8	━	━	H	H
9	━	┈	H	H

RESULTS AND DISCUSSION

Compound **1** was identified as containing a khellactone moiety by the three pairs of typical AB coupling protons at δ 6.24 (1H, d, $J = 9.5$ Hz, H-3) and 7.62 (1H, d, $J = 9.5$ Hz, H-4), 7.37 (1H, d, $J = 8.5$ Hz, H-5) and 6.80 (1H, d, $J = 8.5$ Hz, H-6), 5.40 (1H, d, $J = 4.7$ Hz, H-3′) and 6.59 (1H, d, $J = 4.7$ Hz, H-4′) as well as two methyl singlets at δ 1.47 (3H, s), 1.43 (3H, s) for a *gem*-dimethyl. One acetoxy singlet at δ 2.10 (3H, s) and an angeloyloxy group at δ 6.14 (1H, q, $J = 6.0$ Hz), 1.96 (3H, d, $J = 6.0$ Hz), 1.87 (3H, br.s), suggested a khellactone diester. Correlations of H-3′ with the carbonyl of angeloyloxy group at δ 166.68 and H-4′ with the carbonyl of acetoxy group at δ 170.03 in HMBC decided the ester linkage. Its absolute configuration of (3′*S*)-*O*-angeloyl-(4′*S*)-*O*-acetylkhellactone was confirmed by X-ray crystallographic analysis (Fig. 1). Hydrolysis of **1** afforded **6** and **7**, which were identified by direct comparison of their spectral data with those in the literature [6]. The CD spectrum (Fig. 2) of **6** exhibits a positive Cotton effect at 231 nm, while **7** shows the $\pi\pi^*$ transition of an aromatic structure at 224 nm. Negative Cotton effects at 304 and 310 nm for an $n\pi^*$ transition of the conjugated lactones in the coumarin structure occur for **6** and **7** respectively.

The same methods were employed to determine the structure of **2** as (3′*S*)-*O*-acetyl-(4′-*R*)-*O*-angeloylkhellactone. Its absolute stereochemistry was also confirmed by X-ray crystallographic analysis (Fig. 1). Alkali hydrolysis of **2** lead to the formation of **6** and **7** which were identified by direct comparison with the alkali hydrolysis products of **1** by

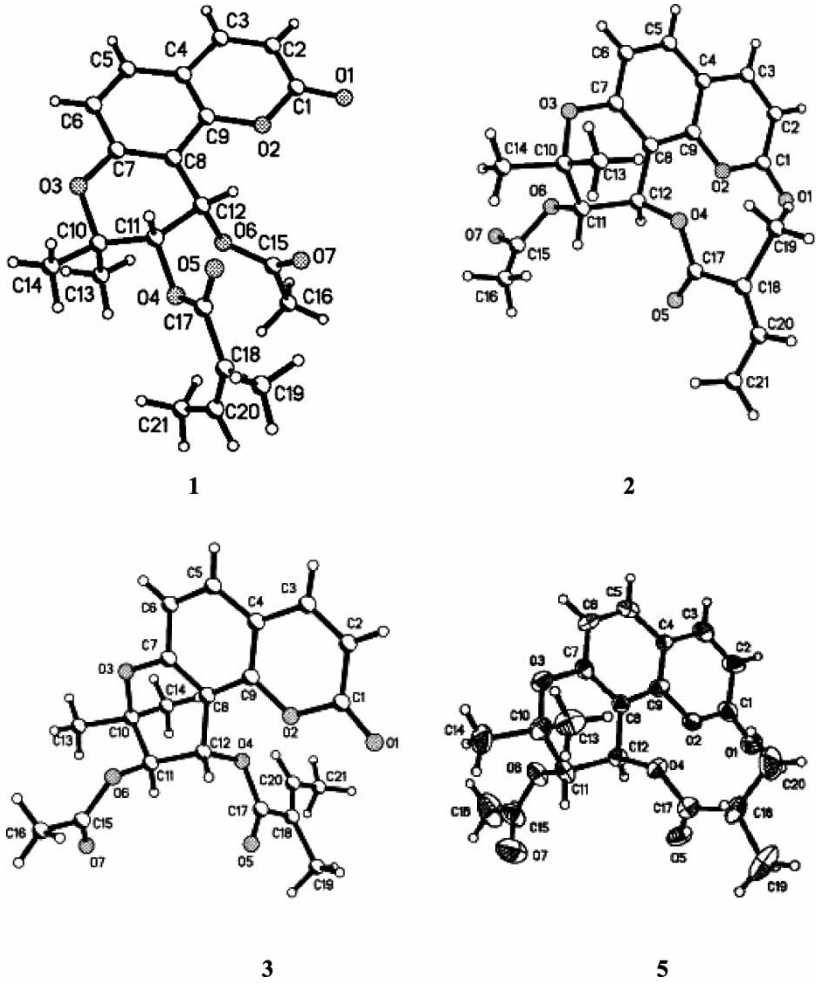

FIGURE 1 X-ray structures of compounds **1**, **2**, **3**, and **5**.

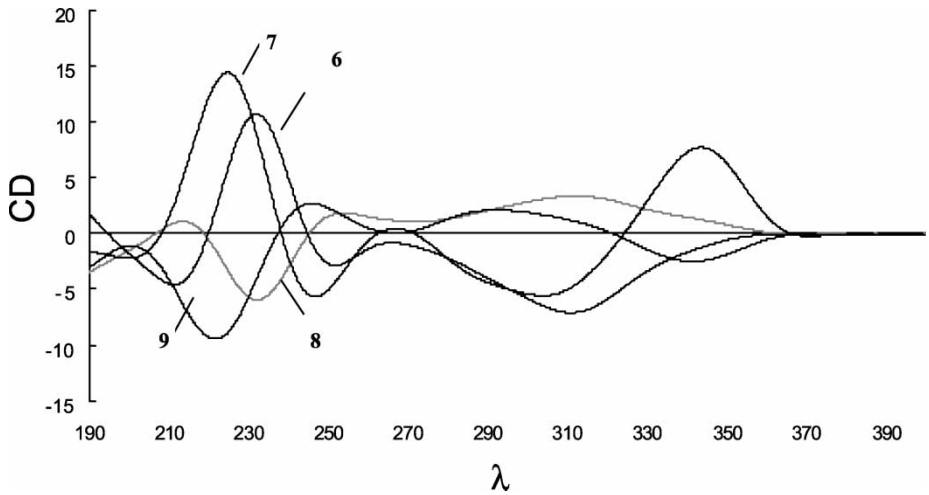

FIGURE 2 CD curves of **6**–**8**.

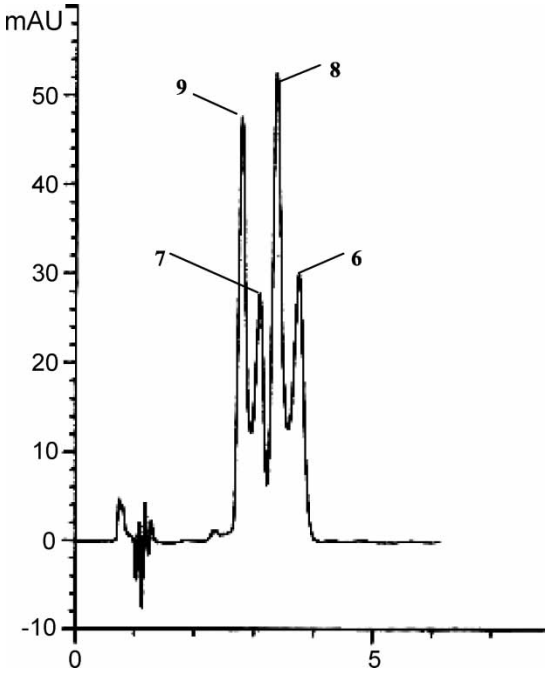

FIGURE 3 HPLC separation of compounds **6**–**9**. Chiral separation of **6**–**9** was achieved with Angilent HP 1100 on an ODS column (4.6 × 150, Dikma, USA). Mobile phase: methanol–acetonitrile–5% β-HCD (3:1:6); Flow rate: 1 ml min; detection at 320 nm.

TABLE I NMR data of compound **3**

No.	*Protons*	*Carbons*
2	6.26 d (9.5)	160.21
3	7.62 d (9.5)	113.70
4		143.55
5	7.40 d (8.5)	129.48
6	6.85 d (8.5)	114.87
7		156.98
8		107.12
9		154.73
10		112.88
2′		77.40
3′	5.35 d (3.4)	71.81
4′	6.25 d (3.4)	63.84
gem-Me	1.48 s	24.17
	1.41 s	24.12
Ac		
1		169.71
2	2.11 s	21.11
Tig		
1		166.78
2		128.60
3	6.83 q (7.0)	138.34
4	1.78 d (7.0)	12.56
5	1.86 br.s	14.81

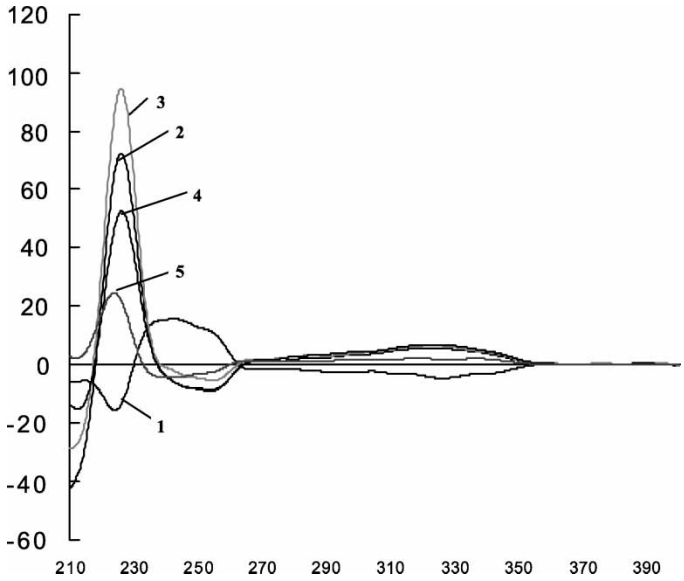

FIGURE 4 CD curves of **1**–**5**.

Rp-18 HPLC (Fig. 3) eluting with methanol–acetonitrle–5% hydroxypropyl-β-cyclodextrin (β-HCD) (3:1:6). Their identical Cotton effects confirmed this elucidation.

Compound **3** was identified as (3′*R*)-*O*-acetyl-(4′-*S*)-*O*-tigloylkhellactone by its ^{1}H and ^{13}C NMR (Table I) spectra. Its absolute configuration was established by X-ray crystallographic analysis (Fig. 1) and measurement of CD curves (Fig. 2) of compounds **8** and **9** formed by alkali hydrolysis of **3**. Contrary to the Cotton effects of **6** and **7**, compounds **8** and **9** exhibit negative Cotton effects at 232 and 222 nm for the $\pi\pi^*$ transition of aromatic structure, and positive effects at 294 and 312 nm for $n\pi^*$ transition of the conjugated lactones in the coumarin.

Compound **4** is a resin solid, its structure was identified as (3′*R*)-*O*-acetyl-(4′*R*)-*O*-angeloylkhellactone by analysis of its ^{1}H and ^{13}C NMR spectral data. Its absolute configuration was confirmed by the formation of compounds **8** and **9**, as determined by CD measurement.

The same methods were used to determine the structure of compound **5**. Its stereostructure was also confirmed by X-ray crystallographic analysis (Fig. 1) as well as by measuring the CD curve of its hydrolysis products **6** and **7**.

To easily establish the stereostructures of angular dihydropyranocoumarin, NMR data are useful in determining the *cis*- or *trans*-configuration at the 3′- and 4′-positions, as summarized previously [9,10]. We investigated the relationship between the absolute stereostructures and the Cotton effects in CD curves. All compounds, except **1**, show positive Cotton effects at around 225 nm in the CD curves (Fig. 4) if the substitute at C_3' is an acetyl group. No direct relationship between the CD curves and the absolute configuration at C_3' or C_4' was found. On the contrary, if the substitute at C_4' was an acetyl, as in compound **1**, the opposite result arose, i.e. a negative Cotton effect at 225 nm. Substituents at C_4' can also change the peak position, as shown in **1** and **5** where the peak absorption moves slightly to shorter wavelength, possibly due to the unconjugated acetyl and isobutyl, respectively, at C_4'. CD measurement of the hydrolyzed products, however, provides a way to decide the absolute configuration of C_3'. If a positive Cotton effect at 220–235 nm appears in CD curves, C_3' has an *S* configuration, while a negative Cotton effect at 220–235 nm indicates an

R configuration at C_3'. The configuration change at C_4' only affects the maximum peak position (Fig. 2).

Stereoisomers can be discriminated by chiral HPLC [11]. Thus the stereoisomers of compounds **6**–**9** were seperated with an Rp-18 column with 5% hydroxypropyl-β-cyclodextrin (β-HCD) (Fig. 3). This provides another easy way to discriminate the configuration if **6**–**9** are available as authentic samples for chiral HPLC.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on a Yanaco micro-melting point apparatus and are uncorrected. UV spectra were taken on a Shimadzu UV 240 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer (600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR). Chemical shifts were given in δ (ppm), based on the TMS. MS was measured with Jeol JM-HX110 mass spectrometer. X-ray structural analysis was made on a Bruker-p4 diffractometer. Preparative HPLC was performed with a Waters 600–996 on a Prepak Cartridge 25 × 100 packed with an ODS column (Waters), with a mixture of methanol and water as mobile phase (5 mL min^{-1}), and detected at 280 nm. Chiral separation was achieved with Angilent HP 1100 on an ODS column (4.6 × 150, Dikma, USA) with methanol–acetonitrle–5% β-HCD (3:1:6) as mobile phase. TLC was performed on precoated aluminum sheets (Rp-18 F_{254}, 0.2 mm, Merck) with MeOH–H_2O (5:5).

Plant Material

Peucedanum praeruptorum Dunn was collected in Zhejiang Province, China in September 2002 and taxonomically identified by Professor Yongyao Li of the School of Pharmaceutical Sciences; a voucher specimen has been deposited at the Herbarium of the same school, Shandong University.

Extraction and Isolation

Fresh root (52 kg) was chopped, extracted with boiling 95% ethanol and concentrated under reduced pressure. The ethanol extract was partitioned with light petroleum ether (boiling point range 60–90°C) to yield a light-petroleum-soluble fraction. Upon work-up of the solvent, a precipitate, which exhibited calcium antagonist effects, formed (68 g) and was filtered off the remaining oil (980 g) after standing at 4°C for 48 h. Recrystallization of the precipitate (5 g) in ethanol afforded compound **1** (3.2 g) and the filtrate was then further separated by preparative HPLC to afford **2** (88 mg), **3** (46 mg), **4** (140 mg) and **5** (32 mg) respectively.

For alkaline hydrolysis, the coumarin ester (1–1.5 mg) was dissolved in dioxane (0.5–1 ml) and added to 0.5 M KOH dropwise. The resultant reaction mixture was then stirred at 60°C for 2 h and the reaction terminated by neutralization with 5% H_2SO_4, followed by extraction with chloroform (2 ml). After evaporation of the solvent, the residue was then either separated by preparative HPLC or directly analyzed by HPLC with solvent containing β-HCD.

(+)-Praeruptorin A (1)

Colorless plates, $[\alpha]_D^{25}$: + 48.2 (MeOH, *c* 0.16), mp 136 ~ 137°C (EtOH). UV λ_{max} (EtOH) (nm): 219, 256, 324. ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data are the same as those of previously reported [1]. CD: $\Delta\varepsilon_{326\,nm,}$ − 1.523, $\Delta\varepsilon_{242.4\,nm}$, + 5.041, $\Delta\varepsilon_{224\,nm}$, − 5.07, Hydrolysis of **1** afforded compound **6** and **7**, as determined by preparative HPLC. Crystal data: $C_{21}H_{22}O_7$, Monoclinic, P21, $a = 9.5682(13)$, $b = 14.956(3)$, $c = 14.2015(19)$ Å, $\beta = 94.052(10)°$, $V = 2027.2(17)$ Å^3, $z = 4$. A total of 8523 reflection were collected using graphite monochromated MoKα radiation at $\lambda = 0.71073$ Å.

Peucedanocoumarin II (2)

Colorless needles, $[\alpha]_D^{25}$: + 8.2 (MeOH, *c* 0.42), mp 124–126°C (decomp.). UV λ_{max} (EtOH) (nm): 208, 220(sh), 255, 322. ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data are the same as those previously reported [7]. CD: $\Delta\varepsilon_{324\,nm}$, + 1.78, $\Delta\varepsilon_{253.6\,nm}$, − 2.934, $\Delta\varepsilon_{225.8\,nm}$, + 23.369. Crystal data: $C_{21}H_{22}O_7$, monoclinic, P21, $a = 9.344(2)$, $b = 11.3424(16)$, $c = 10.6211(17)$ Å, $\beta = 115.149(12)°$, $V = 1018.9(3)$ Å^3, $z = 2$. A total of 3162 reflection were collected using graphite monochromated MoKα radiation at $\lambda = 0.71073$ Å.

(3′R)-O-Acetyl-(4′S)-O-tigloylkhellactone (3)

Colorless plates, $[\alpha]_D^{25}$: + 56.2 (MeOH, *c* 0.26), mp 152–154°C (EtOH). UV λ_{max} (EtOH) (nm): 208, 220(sh), 255, 323. ^{1}H ($CDCl_3$, 600 MHz) δ and ^{13}C NMR ($CDCl_3$, 150 MHz) data are given in Table I. CD: $\Delta\varepsilon_{323\,nm}$, + 2.052, $\Delta\varepsilon_{254.6\,nm}$, − 1.788, $\Delta\varepsilon_{225.8\,nm,}$ + 30.498. Hydrolysis of **3** afforded compound **8** and **9**, as determined by preparative HPLC. Crystal data: $C_{21}H_{22}O_7$, monoclinic, P21, $a = 9.365(2)$, $b = 13.0234(17)$, $c = 16.388(2)$ Å, $\beta = 90°$, $V = 1998.7(6)$ Å^3, $z = 4$. A total of 2788 reflection were collected using graphite monochromated MoKa radiation at $\lambda = 0.71073$ Å.

Pteryxin (4)

Oily resin. $[\alpha]_D^{25}$: + 11.2 (MeOH, *c* 0.12). UV λ_{max} (EtOH) (nm): 203, 220 (sh), 250 (sh), 323. ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data are as same as those previously reported [7]. CD: $\Delta\varepsilon_{324.4\,nm}$, +2.179, $\Delta\varepsilon_{253.8\,nm} = -2.797$, $\Delta\varepsilon_{226\,nm}$, +16.924.

Isobocconin (5)

Colorless plates, $[\alpha]_D^{25}$: + 51.2° (MeOH, *c* 0.16)mp 166–167°C (EtOH). UV λ_{max} (EtOH) (nm): 206, 220(sh), 245, 255, 322. ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data are same with those of previously reported [3]. CD: $\Delta\varepsilon_{336.6\,nm}$, +0.662, $\Delta\varepsilon_{242.6\,nm}$, − 1.423, $\Delta\varepsilon_{223.8\,nm}$, +7.899. Crystal data: $C_{20}H_{22}O_7$, othorhombic, P21, $a = 9.087(5)$, $b = 10.659(5)$, $c = 20.377(5)$ Å, $\beta = 90°$, $V = 1973.7(15)$ Å^3, Crystal size: 0.35 × 0.31 × 0.10 mm, $z = 4$. A total of 2648 reflection were collected using graphite monochromated MoKα radiation at $\lambda = 0.71073$ Å.

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