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Salvigenane and Isosalvipuberulan Diterpenoids from *Salvia leucantha.*

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Abstract.- From the aerial parts of *S. leucantha* the rearranged neo-clerodane diterpenoid salvileucantholide was isolated. Its structure **1** was established by spectral means, chemical transformation and X-ray diffraction analysis. The salvigenane diterpenoid **4** and salvifaricin were also isolated from this source.

INTRODUCTION

In recent years, we have undertaken a systematic chemotaxonomic study of the Mexican Salvia species [1,2], which have been classified in the subgenus Calosphace[3]. Most of the American Salvia species studied up to now[4] contain neo-clerodane diterpenoids or products with rearranged skeletons biogenetically related to a clerodane precursor[5].

In this paper, we describe the diterpenoid constituents of *Salvia leucantha* Cav., a species classified[3] in section Albolanatae, which also includes *S. tomentella*, a species endemic to Brazil.

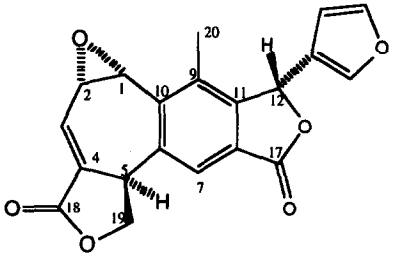
RESULTS AND DISCUSSION

Repeated chromatography of the polar fraction of the acetone extract of the aerial parts of *S. leucantha* led to the isolation of the chalcone isosalipurpol[6], and the known neo-clerodane diterpenoid salvifaricin[7], previously isolated from *S. farinacea* (Sect. Farinaceae). Two new diterpenoids with rearranged skeletons, salvileucantholide **1** and salviandulin E **4**, were also isolated. The structures proposed for these products were established by spectral means, chemical transformations and X-ray diffraction analysis of **1**.

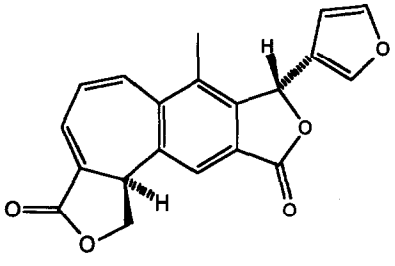
The mass spectrometry of salvileucantholide **1** was consistent with a $C_{20}H_{14}O_6$ molecular formula. The parent peak at m/e 95 suggested the attachment of a lactone group and a furan ring to C-12[8]. The IR spectrum indicated the presence of a furan ring (1595 and 874 cm^{-1}) and α,β-unsaturated γ-lactone (1763 and 1681 cm^{-1}). The UV spectrum (see experimental) agreed with a high degree of unsaturation in the molecule. The proton and carbon resonance spectra were of a great help to establish the structure of salvileucantholide as **1**. Both spectra proved the presence of a β-substituted furan ring in **1** (Tables 1 and 2). COSY experiments revealed a coupling between H-14 and a broad singlet at δ 6.4 which was, therefore, assigned to the γ-lactone closure H-12 (^{13}C 74.9 d). A broad singlet at δ 7.8 was ascribed to an aromatic proton, it showed a long range coupling with the aromatic

methyl broad signal observed at δ 2.3, in COSY experiments. The ^{13}C NMR spectrum (Table 2) indicated the presence of a pentasubstituted aromatic ring in **1**, a doublet at δ 118.0 was assigned to the aromatic C-7. The ^{1}H NMR spectrum of **1** also showed a double doublet (J=4.2 and 2.4 Hz) at δ 6.9 ascribed to the β proton of an α,β–unsaturated γ- lactone it was, therefore, assigned to H-3. The AB part of an ABX system at δ 4.6 (dd, J=10.6 and 7.7 Hz) and 5.2 (dd, J=10.6 and 2.4 Hz) was attributed to the C-19 methylene.The COSY spectrum showed that this methylene was coupled to a signal observed at δ 5.4 as a double triplet (J=7.7 and 2.4 Hz) which was ascribed to H-5. The downfield shift observed for H-5 could be due to the deshielding effect produced by the disubstituted oxirane group, whose existence was confirmed by the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2). A doublet at δ 4.8 (J=4.2 Hz) was assigned to H-1 while a triplet at δ 4.1 (J=4.2 Hz) to H-2. COSY experiments showed that H-2 was coupled to H-1 and to H-3. Two carbon doublets at δ 55.3 and 52.1 were attributed to the oxirane carbons 1 and 2.

The structure **1** proposed for salvileucantholide is closely related to that of the benzocycloheptatriene diterpenoid, isosalvipuberulin **3** previously isolated from *Salvia puberula* (sect. Holwaya)[8]. In order to prove this structural relationship, salvileucantholide **1** was subjected to treatment with sodium iodide-*p*-toluensulfonic acid in acetonitrile[7]. The ^{1}H NMR spectrum of the product obtained **2**, showed that the oxirane group was replaced by a double bond but the expected isomerization to **3** was not produced. The presence of the 1,2-double bond in desoxysalvileucantholide **2** was established by the ^{1}H NMR spectrum which showed a doublet at δ 7.5 (J=12 Hz) and a double doublet at δ 7.0 (J=12 and 5 Hz). A broad doublet at δ 3.6 was attributed to H-5. The upfield shift



1



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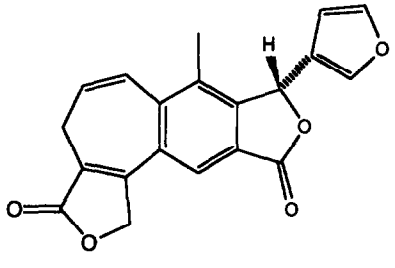

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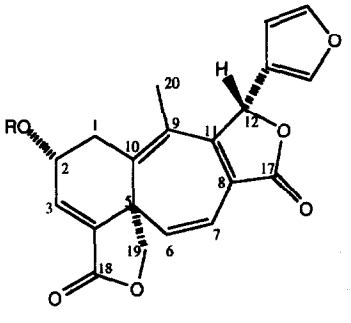


4. R = H

5. R = Ac

Table 1. ^{1}H NMR data for compounds **1-2, 4-5** (200 MHz, $CDCl_3$, TMS.)

H	1	2&	4#	5
1	4.8 *d* (4.2)	7.5 *d* (12)	α 2.3 *br dd* (15.6, 10.0)	α 2.4 *m*
			β 2.9 *dd* (15.6, 5.0)	β 3.1 *dd* (14, 5.1)
2	4.1 *t* (4.2)	7.0 *dd* (12, 5)	4.1 *ddd* (10.0, 5.0, 1.7)	5.3 *ddd* (9.9, 5.1, 2.1)
3	6.9 *dd* (4.2, 2.4)	7.1 *dd* (5, 2)	7.1 *d* (1.7)	7.3 *d* (2.1)
5	5.4 *dt* (7.7, 2.4)	3.6 *br d* (8)	-	-
6			5.5 *d* (9.1)	5.5 *d* (9.0)
7	7.8 *br s*	7.85 *s*	6.8 *dd* (9.1, 1.4)	6.9 *dd* (9.0, 1.3)
12	6.4 *br s*	6.40 *s*	6.6 *d* (1.4)	6.3 *d* (1.3)
14	6.1 *dd* (1.5, 0.9)	6.1 *dd* (1.5, 0.9)	6.5 *dd* (1.7, 0.7)	6.2 *m*
15	7.4 *t* (1.5)	7.4 *t* (1.5)	7.7 *t* (1.7)	7.4 *t* (1.7)
16	7.6 *br s*	7.6 *br s*	8.0 *d* (0.7)	7.6 *br s*
19A	4.6 *dd* (10.6, 7.7)	4.8 *dd* (10.6, 8.0)	3.2 *d* (8.4)	3.3 *d* (8.4)
19B	5.2 *dd* (10.6, 2.4)	5.3 *dd* (10.6, 2.8)	3.8 *d* (8.4)	3.7 *d* (8.4)
3H-20	2.3 *br s*	2.25 *s*	2.1 *d* (2.8)	2.1 *d* (2.8)
CH_3CO-	-	-	-	2.2 *s*

Run in DMSO-d_6. &Run in $CDCl_3$:DMSO-d_6 (1:1) mixture.

Table 2. ^{13}C NMR data for compounds **1** and **4** (50 MHz, TMS).

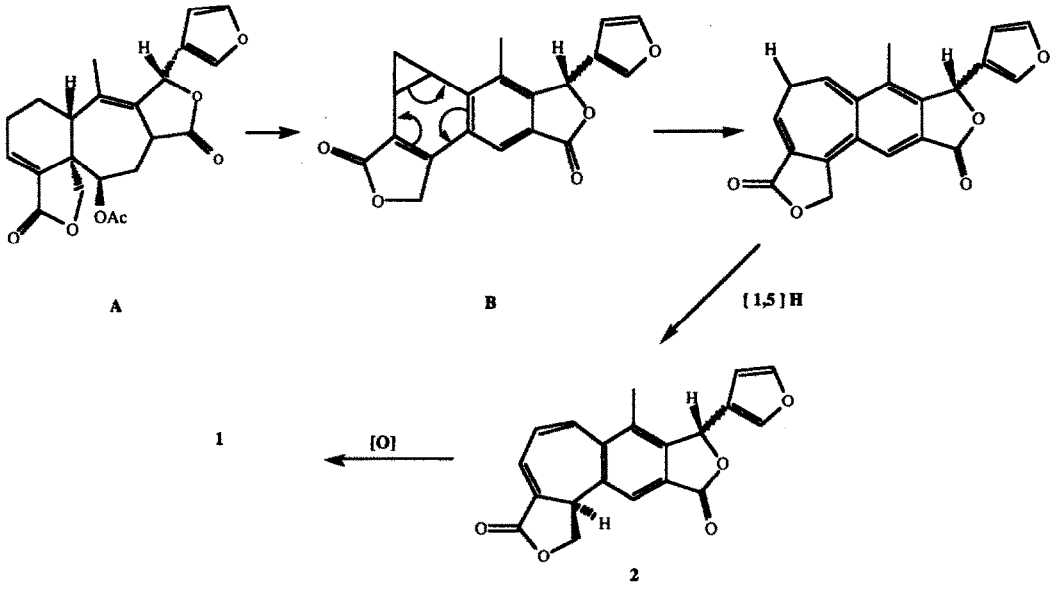
C	1*	4#	C	1	4
1	55.3 *d*	37.9 *t*	11	130.5 *s*	159.4 *s*
2	52.1 *d*	66.6 *d*	12	74.9 *d*	75.6 *d*
3	130.6 *d*	144.5 *d*	13	120.9 *s*	120.5 *s*
4	134.9 *s*	130.9 *s*	14	108.8 *d*	109.0 *d*
5	37.1 *d*	46.2 *s*	15	144.5 *d*	144.2 *d*
6	146.4 *s*	129.2 *d*	16	142.2 *d*	142.3 *d*
7	118.0 *d*	120.9 *d*	17	168.2 *s*	171.2 *s*
8	126.5 *s*	124.5 *s*	18	169.6 *s*	168.0 *s*
9	136.9 *s*	125.9 *s*	19	66.9 *t*	74.2 *t*
10	137.2 *s*	131.7 *s*	20	15.8 *q*	16.4 *q*

*Run in $CDCl_3$. #Run in $CDCl_3$ + DMSO-d_6 (1:1). Multiplicities obtained by DEPT pulse sequence and confirmed by HETCOR.

of this signal as compared with the chemical shift of H-5 in salvileucantholide (δ 5.4) proved that the epoxy oxygen and H-5 are in a close spatial arrangement. The rest of the spectrum was very similar to that of salvileucantholide (Table 1). Treatment of **2** with trifluoroacetic acid in the NMR tube produced some isomerization to isosalvipuberulin **3** (30% of **3** by ^{1}H integration) as shown by the appearance of a strong singlet at δ 5.5 which could be due to the C-19 methylene[8].

The relative stereochemistry of salvileucantholide could not be established by spectral analysis. In order to prove the structure proposed and establish the relative configuration of the chiral centres in **1**, an X-ray diffraction analysis of a single crystal was undertaken. The molecule displays a stepped conformation, with the twisted γ-lactone ring D (*pseudo* two-fold axis through C-18) lying on the β face of the planar benzofuranone moiety and the nearly perpendicular (angle between planes: 111.8°) furan ring E on the opposite side (Fig 1b). The seven-membered ring A adopts a boat conformation, which places the H-5 hydrogen atom just in front of the O-3 oxygen atom of the oxirane group leading to a very strong *trans*-annular interaction [H-5 ••• O-3; 2.36(8)Å]. This fact explains in turn, the great high-field shift observed in the ^{1}H-NMR spectrum for the signal of this proton. The stereochemistry shown in Figure 1a, is relative to H-12 β.

The isosalvipuberulan structure **1** established for salvileucantholide could be biogenetically derived from salvigenolide **A**, a diterpenoid isolated from *S. fulgens* (Sect. Fulgentes)[9] as had been previously proposed[8] for the biogenesis of isosalvipuberulin **3**. Thus, loss of the C-6 acetoxy group followed by a 6→7 expansion of the



Scheme 1. Proposed biogenetic pathway to salvileucantholide **1**.

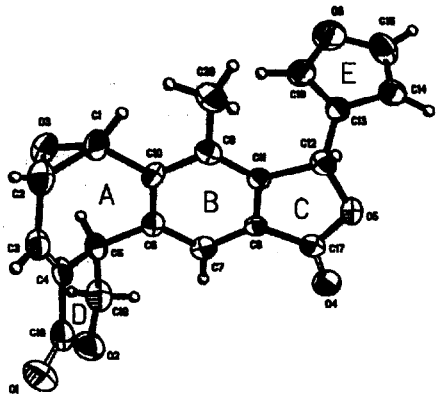


Figure 1a. The molecular structure of the title compound (**1**) showing the atom labelling. Thermal ellipsoids are drawn at 50% probability level.

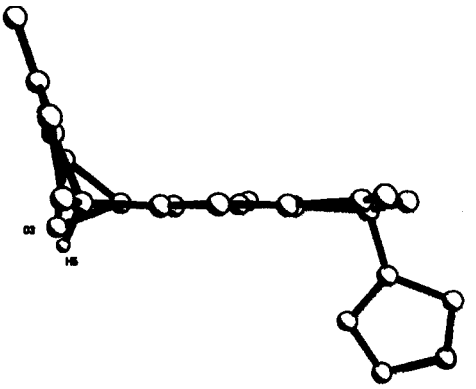


Figure 1b. Side view of the molecule showing its conformation and the *trans*-annular interaction between H-5 and O-3

electrocyclic reaction of **B** followed by a suprafacial 1,5-sigmatropic hydrogen shift to **2** and epoxydation of the 1,2 double bond, could give rise to salvileucantholide **1** (Scheme 1).

The second rearranged diterpenoid **4** isolated from *S. leucantha* proved to have a highly unsaturated salvigenane structure. It had been previously found in *S. lavanduloides* (Sect. Lavanduloideae)[10] and was named salviandulin E. Salviandulin E had a mass spectrum consistent with a $C_{20}H_{16}O_6$ molecular formula. The formation of the base peak at m/e 95 suggested that a furan and a lactone groups were bound to C-12 as in **1**. The UV spectrum (see experimental) confirmed the highly unsaturated nature of **4**. The IR spectrum showed bands due to hydroxy group (3440 cm^{-1}), α, β–unsaturated γ-lactone (1754 and 1630 cm^{-1}) and the characteristic furan bands (1603, 1536, 1505 and 874 cm^{-1}).

The ^1H and ^{13}C NMR spectra (Tables 1 and 2) confirmed the presence of a β substituted furan ring in **4**. The ^1H NMR spectrum presented a doublet at δ 5.5 (J=9.1 Hz) and a double doublet at δ 6.8 (J=9.1 and 1.4 Hz) which were assigned to the vinylic protons 6 and 7. The small coupling constant observed for H-7 proved to be due to long range coupling with H-12 by double resonance experiments. Thus irradiation of the doublet (J=1.4 Hz) at δ 6.6 transformed the double doublet ascribed to H-7 into a doublet (J=9.1 Hz). The COSY spectrum confirmed the assignment of the H-12 signal at δ 6.6 and showed the expected interaction of this signal with the furan protons and with H-7. It also showed a correlation between a vinylic methyl doublet (δ 2.1, J=2.8 Hz) ascribed to Me-20 and a broad double doublet at δ 2.3 attributed to H-1α (Table 1). A double doublet at δ 2.9 was unambiguously assigned to H-1β. The ^1H NMR spectrum of salviandulin E **4**, also showed an AB system at δ 3.2 and 3.8 (two doublets, J=8.4 Hz) ascribed to the 19 methylene protons of the α,β-unsaturated 18,19-olide

group. A doublet at δ 7.1 (J=1.7 Hz) was attributed to the vinylic H-3 and was shown to be coupled to the geminal proton of an hydroxyl α–equatorially bound to C-2, which was observed at δ 4.1 (ddd, J=10.0, 5.0 and 1.7 Hz) by double resonance experiments.

The ^{13}C NMR spectrum of salviandulin E (Table 2) confirmed the structure **4** proposed for it. The multiplicities were obtained by DEPT pulse sequence. The assignments of the hydrogen bearing carbon resonances were established by HETCOR experiments. It is interesting to note that only one sp^3 triplet and one sp^3 singlet of no oxygen bound carbon atoms were observed in the spectrum of **4**, they were unambiguously assigned to C-1 and C-5 respectively. The resonances of the carbonyls and of the sp^2 singlets were assigned by COLOC experiments. They showed correlation between the methyl group and C-9, C-10 and C-11; of H-6 with C-8 and of H-7 with C-11, among others.

Acetylation of salviandulin E under normal conditions yielded the monoacetyl derivative **5** whose IR spectrum did not show hydroxyl absorption. In the ^1H NMR spectrum (Table 1) the acetoxy geminal proton, H-2, was observed at δ 5.3 (ddd, J=9.9, 5.1 and 2.1 Hz) and the acetoxy methyl at δ 2.2 (s). The rest of the spectrum was almost identical to that of the parent compound.

Salviandulin E **4**, can be considered to have a salvigenane skeleton[5]. It constitutes the second diterpenoid isolated from a natural source with this type of carbon arrangement.

It is interesting to draw attention to the isolation of a salvigenane and a isosalvipuberulan diterpenoids from the same Salvia species. This fact could have chemotaxonomic interest and could suggest a botanical relation between sections Fulgentes, Holwaya and Albolanatae of *Salvia*, subgenus Calosphace.

EXPERIMENTAL

Mps. are uncorrected. Plant material was collected in August 1991 in Tehuacan Valley, State of Puebla (México) and a voucher specimen (MEXU 544951) was deposited in the Herbarium of the Instituto de Biología de la UNAM. COSY experiments were obtained at room temperature, using the standard pulse sequence as implemented in the spectrometer (Varian Gemini 200) software.

Extraction and isolation of the constituents from S. leucantha.. Dried and powdered aerial parts of *S. leucantha* (820 g) were extracted with Me_2CO (10 l) at room temperature for four days. The extract (25.3 g) was subjected to partition between MeOH-H_2O (4:1) and benzene-petrol (1:1), to yield 16.45 g of a polar phase which was subjected to vacuum chromatography over silica gel. Mixtures of petrol-EtOAc, of increasing polarity, were used as eluents. From the fractions eluted with petrol-EtOAc (6:4), salvifaricin (12.3 mg) was isolated. The fractions eluted with petrol-EtOAc (6:4) provided 55 mg of salvileucantholide (**1**).The chalcone isosalipurpol (240 mg) was isolated from the same polarity. Salviandulin E (**4**) (231 mg) was obtained as an amorphous solid from the fractions eluted with petrol-EtOAc (4:6).

Salvileucantholide (**1**). Mp. 188-190°C (from Me_2CO). $[\alpha]_D$ = -122.5° (pyridine *c* 0.2). UV (MeOH) λ_{max} nm (log ε) = 205 (4.45), 232 (4.0); IR ($CHCl_3$) ν_{max} cm^{-1}:1763, 1681, 1595, 874; ^1H NMR: Table 1; ^{13}C NMR: Table 2; EIMS m/z (int. rel.): 350 [M^+] (59.4), 348 (7.69), 332 (26.9), 322 (15.4), 321 (34.6), 306 (15.4),

282 (46.1), 277 (17.3), 255 (53.8), 254 (30.8), 253 (17.3), 227 (28.8), 211 (25), 189 (23.1), 178 (26.9), 165 (30), 153 (15.4), 152 ((26.9), 151 (13.5), 141 (11.5), 139 (19.2), 115 (25), 95 (100), 89 (17.3), 83 (15.4), 69 (15.4), 63 (17.32), 57 (19), 55 (23), 43 (30.77). $C_{20}H_{14}O_6$ requires [M^+] at m/z 350.

Salviandulin E. (**4**) Mp. 150-155°C; $[\alpha]_D$ = -36.66° (MeOH, *c* 0.6); UV (MeOH) λ_{max} nm (log ε): 267 (4.9); IR (Nujol) ν_{max} cm^{-1}: 3440, 1754, 1687, 1641, 1630, 1603, 1536, 1505, 874; ^1H NMR: Table 1; ^{13}C NMR: Table 2; EIMS m/z (rel. int.): 352 [M+] (69.8), 351 (56.1), 337 (28.7), 334 (29.9), 306 (27.4), 294 (15), 279 (18.7), 277 (19.9), 265 (23.7), 239 (36.15), 228 (54.9), 198 (67.3), 189 (48.6), 179 (29.9), 165 (75), 143 (60), 141 (48.6), 139 (61.1), 128 (39.9), 115 (74.3), 95 (100), 89 (17.5), 68 (31.16). $C_{20}H_{16}O_6$ requires [M^+] at m/z 352.

Treatment of Salvileucantholide (**1**) *with NaI/pTsOH.* Salvileucantholide (**1**)(15 mg) in dry CH_3CN (1 ml), was treated with NaI (32 mg), and p-toluensulfonic acid (22 mg) under argon atmosphere at room temp. for 20 min. After usual work-up 12.1 mg of the unstable compound (**2**) were obtained. Mp. 155-156°C (from CH_2Cl_2); IR ($CHCl_3$) ν_{max} cm^{-1}: 1761, 1657, 1602, 872; ^1H NMR: Table 1.

Acetylation of Salviandulin E (**4**). Salviandulin E (**4**) (23.8 mg) in pyridine (0.5 ml) was treated with Ac_2O (0.5 ml) at room temp. for 80 min. After usual work-up compound (**5**) (4.4 mg) was obtained. Mp. 124-128°C (from Me_2CO-*iso*-propyl ether); IR ($CHCl_3$)ν_{max} cm^{-1}:1764, 1506, 1026, 909, 873; ^1H NMR: Table 1.

X-Ray structure determination of Salvileucantholide (**1**).*Crystal Data*: empirical formula: $C_{20}H_{14}O_6$; formula weight: 350.3 amu; crystal color and habit: colorless, prism; crystal dimensions (mm): 0.48 x 0.40 x 0.28; crystal system: orthorhombic; space group: $P2_12_12_1$; Z: 4. Unit cell dimensions from 25 Reflections (4.43 ≤2θ ≤ 25.08°) *a*: 9.215(3) ; *b*: 11.743(2); *c*: 14.573(8)Å; volume: 1577.0(8)$Å^3$; density(calc): 1.475 gcm^{-3}; F(000): 728 electrons; linear absorption coefficient μ(MoKα): 0.11 mm^{-1}.

Data collection:: diffractometer Siemens P4/PC; radiation type: MoKα, λ=0.71073Å, graphite monochromator; room temperature; Lp corrections, scan type ω scans; scan range (2θ): 3-50 degrees; octants used: *+h*, *+k*, *+l* (*h*: -1/10; *k*: -1/13; *l*: -1/17) ; scan rate: variable (min./max.: 10.0/30.0 deg. per min.); scan width (ω): 0.60°; standard reflections: 3 measured every 97 reflections; linear decay = 1.2%; number of data collected: 2133; number of unique reflections: 1970; R_{int} = 1.86%.

Solution and Refinement: system used: Siemens SHELXTL/PC[11]; solution: direct methods; refinement method: full-matrix least-squares; function minimized: $\sum|Fo-Fc|^2$; hydrogen atoms: riding model, d_{C-H}=0.96Å, common fixed isotropic U=0.06$Å^2$; reflections used in refinement [F ≥ 3σ(F)]: 1432; number of parameters refined: 236; data/parameter ratio:6.1:1; R=$\sum(|Fo-Fc|)/\sum(|Fo|)$:0.057;Rw=$[\sum(w|Fo-Fc|)^2/\sum w(Fo)^2]^{1/2}$: 0.060; $w^{-1}=\sigma^2(Fo)+0.0005(Fo)^2$; GOF: 1.35; max. shift/error: 0.001; secondary extinction parameter χ= 0.0033(4) ($F^*=F[1+0.002\chi F^2/\sin(2\theta)]^{-1/4}$); residual electron density: 0.48/-0.33 e/$Å^3$.

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