

# Voatinggine and Tabertinggine, Pentacyclic Indole Alkaloids Derived from an Iboga Precursor via a Common Cleavamine-Type Intermediate

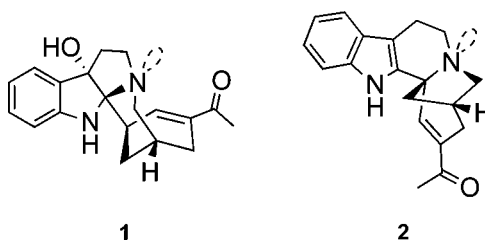
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## ABSTRACT



Two new indole alkaloids, voatinggine (1) and tabertinggine (2), which are characterized by previously unencountered natural product skeletons, were isolated from a Malayan *Tabernaemontana* species. The structures and absolute configuration of these alkaloids were determined using NMR and MS analysis, and X-ray diffraction analysis. A possible biogenetic pathway to these novel alkaloids from an iboga precursor, and via a common cleavamine-type intermediate, is presented.

The genus *Tabernaemontana* (Apocynaceae) incorporates a large number of species which are distributed mainly over tropical America, Africa, and Asia.<sup>1</sup> Plants of the genus are rich sources of alkaloids and are especially known to elaborate structurally novel, as well as biologically active, indole and bisindole alkaloids.<sup>2</sup> Recent examples of such alkaloids isolated from the Malayan members of this genus include the hexacyclic alkaloid tronoharine,<sup>3a</sup> the pentacyclic indole tronocarpine,<sup>3b</sup> the quinolinic alkaloid,

voastrictine,<sup>3c</sup> the hexacyclic indole conolutinine,<sup>4a</sup> the iboga–lignan conjugate, conoliferine,<sup>4b</sup> the vobasiny–iboga bisindoles, conodiparines A–D, which reverse multidrug-resistance in vincristine-resistant KB cells,<sup>5</sup> and the *Aspidosperma*–*Aspidosperma* bisindole conophylline,<sup>6</sup> which was found to be a potent inhibitor of the *ras* function.<sup>7</sup> The last bisindole alkaloid is also notable for its ability to induce  $\beta$ -cell differentiation and to stimulate insulin production in rat pancreatic acinar carcinoma cells<sup>8a</sup> and in cultured fetal rat pancreatic tissue.<sup>8b</sup> In view

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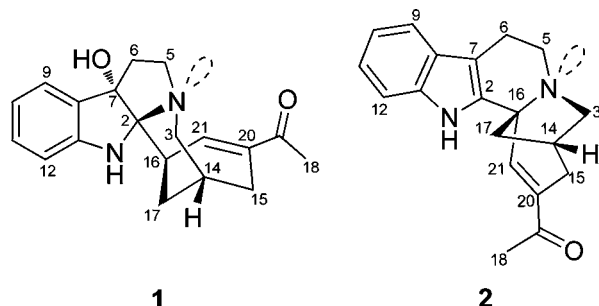
(6) (a) Kam, T. S.; Loh, K. Y.; Lim, L. H.; Loong, W. L.; Chuah, C. H.; Chen, W. *Tetrahedron Lett.* **1992**, *33*, 969–972. (b) Kam, T. S.; Loh, K. Y.; Chen, W. *J. Nat. Prod.* **1993**, *56*, 1865–1871. (c) Kam, T. S.; Pang, H. S.; Lim, T. M. *Org. Biomol. Chem.* **2003**, *1*, 1292–1297.

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of its important biological activities, its total synthesis has also been recently reported.<sup>9</sup> In continuation of our studies of biologically active alkaloids from Malaysian members of this genus, we now report the isolation of two new indole alkaloids, voatinggine (**1**) and tabertingine (**2**), both of which are characterized by an unprecedented skeleton and which are possibly derived from a common cleavamine-type precursor.<sup>10</sup>



Voatinggine (**1**) was initially obtained as a light yellowish oil and subsequently crystallized from  $\text{CH}_2\text{Cl}_2$ –hexanes as colorless block crystals, mp 186–188 °C, with  $[\alpha]_{\text{D}}^{25} +136$  ( $\text{CHCl}_3$ ,  $c$  0.49). The IR spectrum showed bands due to  $\text{NH/OH}$  ( $3391\text{ cm}^{-1}$ ) and conjugated ketone carbonyl ( $1650$ ,  $1611\text{ cm}^{-1}$ ) functions, while the UV spectrum showed dihydroindole absorption maxima at 210, 239, and 297 nm.<sup>11</sup> The ESIMS showed an  $[\text{M} + \text{H}]^+$  peak at  $m/z$  311, and HRESIMS measurements ( $[\text{M} + \text{H}]^+$  311.1765) established the molecular formula as  $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_2$ .<sup>12</sup>

The  $^1\text{H}$  NMR data (Table 1) showed the presence of an unsubstituted indoline chromophore from the presence of four aromatic resonances ( $\delta$  6.58–7.24), an indolic NH ( $\delta$  4.35), an olefinic hydrogen ( $\delta$  7.25, d,  $J = 6\text{ Hz}$ ), and an acetyl side chain ( $\delta_{\text{H}}$  2.32,  $\delta_{\text{C}}$  25.5, 200.3). The  $^{13}\text{C}$  NMR data (Table 1) showed a total of 19 carbon resonances, comprising one methyl, five methylene, seven methine, and six quaternary carbon atoms. The  $^{13}\text{C}$  NMR spectrum confirmed the presence of the conjugated ketone carbonyl ( $\delta_{\text{C}}$  200.3) and a trisubstituted double bond associated with the olefinic hydrogen ( $\delta_{\text{C}}$  141.2, 144.0).

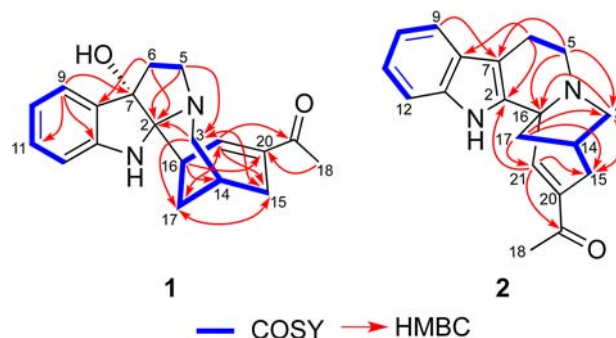
In addition, a deshielded quaternary carbon resonance at  $\delta_{\text{C}}$  89.7 indicated oxygen substitution at this carbon,<sup>4a</sup> while another at  $\delta_{\text{C}}$  92.2 is due to a quaternary carbon linked to two nitrogen atoms.<sup>13</sup> The resonance at  $\delta_{\text{C}}$  89.7 was assigned to C-7 from the observed three-bond correlation from the aromatic doublet at  $\delta$  7.24 to this carbon in the HMBC spectrum. This doublet was in turn assigned to H-9 from its observed reciprocal NOE with H-6 $\beta$  ( $\delta$  2.10), while the other aromatic doublet at  $\delta$  6.58 was due to H-12 from its NOE with the indolic NH.

The COSY spectrum indicated, in addition to the presence of four contiguous aromatic hydrogens, the presence

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Spectroscopic Data of Voatinggine (**1**) and Tabertingine (**2**)

<b>1</b>			<b>2</b>		
H/C	$\delta_{\text{C}}^a$	$\delta_{\text{H}}^b$	H/C	$\delta_{\text{C}}^{a,c}$	$\delta_{\text{H}}^b$
2	92.2		2	135.6	
3 $\alpha$	53.9	2.77 d (11)	3 $\beta$	61.1	3.03 ddd (11, 7, 1)
3 $\beta$		2.91 dd (11, 2)	3 $\alpha$		3.12 d (11)
5 $\beta$	49.3	2.50 td (8, 6)	5 $\beta$	51.2	2.89 td (10, 3)
5 $\alpha$		2.73 t (8)	5 $\alpha$		3.09 m
6 $\alpha$	42.4	1.94 td (12, 8)	6 $\beta$	22.2	2.80 dt (15, 3)
6 $\beta$		2.10 dd (12, 6)	6 $\alpha$		2.95 m
7	89.7		7	108.3	
8	132.2		8	126.5	
9	123.4	7.24 d (8)	9	118.5	7.54 dd (8, 1)
10	118.8	6.77 t (8)	10	119.5	7.15 ddd (8, 7, 1)
11	129.4	7.11 t (8)	11	121.8	7.21 ddd (8, 7, 1)
12	109.3	6.58 d (8)	12	111.2	7.36 dt (8, 1)
13	148.5		13	136.6	
14	26.8	2.15 m	14	33.6	2.84 m
15 $\beta$	30.9	2.26 d (19)	15 $\alpha$	33.1	2.37 d (18)
15 $\alpha$		2.56 dd (19, 7)	15 $\beta$		2.50 d (18)
16	36.8	2.67 m	16	60.7	
17 $\alpha$	27.0	1.69 d (13)	17 $\alpha$	35.8	1.94 d (11)
17 $\beta$		1.86 d (13)	17 $\beta$		2.07 dd (11, 5)
18	25.5	2.32 s	18	25.3	2.26 s
19	200.3		19	200.8	
20	141.2		20	138.7	
21	144.0	7.25 d (6)	21	145.7	6.85 s
NH		4.35 br s	NH		7.89 br s

<sup>a</sup>  $\text{CDCl}_3$ , 100 MHz. <sup>b</sup>  $\text{CDCl}_3$ , 600 MHz. <sup>c</sup>  $\text{CDCl}_3$  with few drops of  $\text{CD}_3\text{OD}$  added.



**Figure 1.** COSY and HMBC data of **1** and **2**.

of  $\text{NCH}_2\text{CH}_2$  and  $\text{NCH}_2\text{CH}(\text{CH}_2)\text{CH}_2\text{CHCH}$  partial structures. The  $\text{NCH}_2\text{CH}_2$  fragment corresponds to N-4–C-5–C-6 from the observed H-6 to C-8, C-2, and H-5 to C-2, C-3, three-bond correlations in the HMBC spectrum. This also confirmed the assignment of the low-field quaternary resonance at  $\delta_{\text{C}}$  92.2 to C-2, which is linked to both the indolic N-1 and N-4, and indicated attachment of the C-5–C-6 fragment to the hydroxy-substituted C-7 at  $\delta_{\text{C}}$  89.7. These observations reveal the fusion of the pyrrolidine ring unit to the indole moiety at

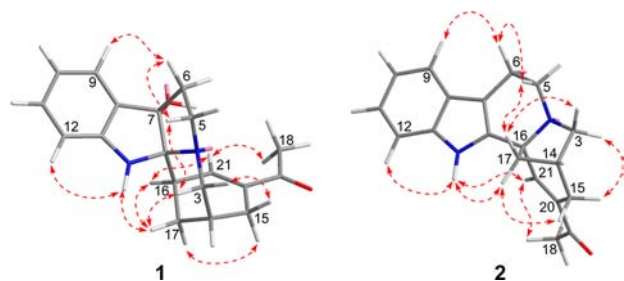
(10) Plant material (*T. corymbosa*) was collected in Johor, Malaysia, and was identified by Dr. Richard C. K. Chung, Forest Research Institute, Malaysia.

(11) Sangster, A. W.; Stuart, K. L. *Chem. Rev.* **1965**, *65*, 69–130.

(12) HRESIMS found  $m/z$  311.1765 (calcd for  $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_2 + \text{H}$ , 311.1754).

(13) Gan, C. Y.; Low, Y. Y.; Thomas, N. F.; Kam, T. S. *J. Nat. Prod.* **2013**, *76*, 957–964.

C-2 and C-7. The  $\text{NCH}_2\text{CH}(\text{CH}_2)\text{CH}_2\text{CHCH=}$  partial structure can be considered as the sum of two fragments sharing a common branching point (i.e., C-14). The  $\text{NCH}_2\text{CHCH}_2$  fragment corresponds to N-4–C-3–C-14–C-15, while the  $\text{NCH}_2\text{CHCH}_2\text{CHCH=}$  fragment corresponds to N-4–C-3–C-14–C-17–C-16–C-21. These assignments were supported by the observed H-3 to C-15, C-17, H-17 to C-15, and H-15 to C-3, three-bond correlations in the HMBC spectrum (Figure 1). The attachment of the C-18–C-19 acetyl side chain at the olefinic C-20 was indicated by the observed three-bond correlations from H-18 to C-20 and from H-21 to C-19, while the observed correlations from H-16 to C-14, C-20, and from H-21 to C-15, C-17, established the  $\alpha,\beta$ -unsaturated cyclohexene ring moiety with acetyl side chain substitution at C-20. Completion of the assembly required the linking of C-16 to C-2 to forge the pentacyclic diazaspino structure of voatinggine. The presence of the diazaspino C-2 carbon was supported by its observed carbon shift at  $\delta_{\text{C}}$  92.2.<sup>13</sup>

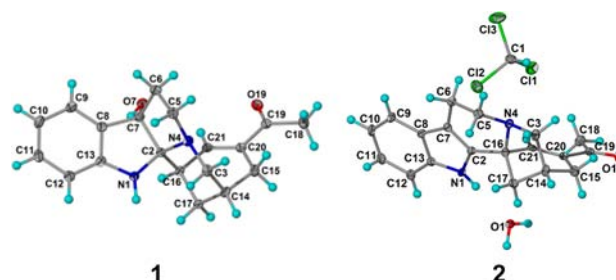


**Figure 2.** Selected NOEs of **1** and **2**.

The proposed structure is in complete accord with the HMBC data (Figure 1). The structure is also in accord with the NOE data (Figure 2), which also confirmed the relative configuration at the various stereogenic centers. In the event, since suitable crystals were eventually obtained from dichloromethane–hexane solution, the proposed structure and absolute configuration<sup>14</sup> were confirmed by an X-ray diffraction analysis (Figure 3).<sup>15</sup>

Another alkaloid, tabertinggine (**2**) with a previously unencountered natural product skeleton and biogenetically related to voatinggine (**1**) (vide infra), was also isolated from the bark extract. Tabertinggine (**2**) was initially obtained as an amorphous solid, which subsequently crystallized from chloroform as colorless needles, mp 113–115 °C, with  $[\alpha]_{\text{D}}^{25} +107$  ( $\text{CHCl}_3$ ,  $c$  0.40). The IR spectrum showed bands due to NH ( $3466\text{ cm}^{-1}$ ) and conjugated ketone carbonyl ( $1667$ ,  $1628\text{ cm}^{-1}$ ) functions. The UV spectrum

showed characteristic indole absorption maxima at 225, 282, and 289 nm.<sup>11</sup> The ESIMS showed an  $[\text{M} + \text{H}]^+$  peak at  $m/z$  293, and HRESIMS measurements ( $[\text{M} + \text{H}]^+$  293.1653) established the molecular formula as  $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}$ .<sup>16</sup>



**Figure 3.** X-ray crystal structures of **1** and **2**.

The  $^1\text{H}$  NMR data of **2** (Table 1) shared several features, which were also common in the spectrum of **1**, such as the presence of an unsubstituted indole chromophore from the presence of four aromatic resonances ( $\delta$  7.15–7.54), an indolic NH ( $\delta$  7.89), an isolated olefinic hydrogen (singlet at  $\delta$  6.85), and an acetyl side chain ( $\delta_{\text{H}}$  2.26,  $\delta_{\text{C}}$  25.3, 200.8). The  $^{13}\text{C}$  NMR data (Table 1) showed a total of 19 carbon resonances, comprising one methyl, five methylene, six methine, and seven quaternary carbon atoms. In common with **1**, the  $^{13}\text{C}$  NMR spectrum showed the presence of the conjugated ketone carbonyl ( $\delta_{\text{C}}$  200.8) and a trisubstituted double bond associated with the olefinic hydrogen ( $\delta_{\text{C}}$  138.7, 145.7). However, unlike **1**, the deshielded hydroxy-substituted quaternary resonance at  $\delta_{\text{C}}$  89.7 (C-7), and the downfield quaternary carbon resonance at  $\delta_{\text{C}}$  92.2 resonance (C-2) attributed in **1** to its attachment to two nitrogen atoms, were absent in the spectrum of **2**. These signals were replaced in the spectrum of **2** by  $\text{sp}^2$  indole carbon resonances at  $\delta_{\text{C}}$  108.3 (C-7) and 135.6 (C-2) respectively. Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **1** and **2** showed that a methine in **1** has been replaced by a quaternary carbon in **2**, which was eventually traced to C-16 ( $\delta_{\text{H}}$  2.67,  $\delta_{\text{C}}$  36.8 in **1**;  $\delta_{\text{C}}$  60.7 in **2**). Since C-16 is adjacent to the indole moiety, the low field resonance of this quaternary carbon suggested that it is also linked to a nitrogen atom, which turned out to be the case (vide infra).

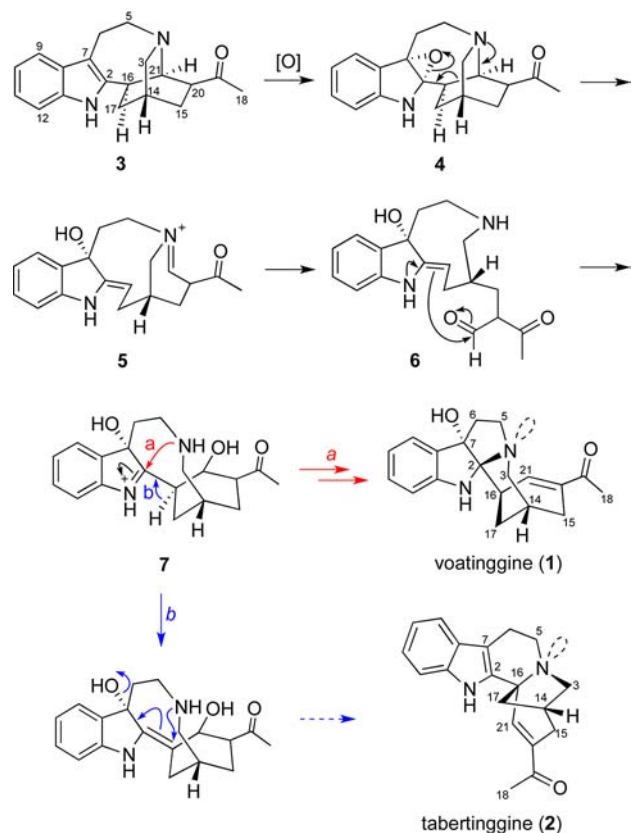
As in the case of **1**, the COSY spectrum also indicated in addition to the presence of the four contiguous aromatic hydrogens, the presence of  $\text{NCH}_2\text{CH}_2$  which is attributed to N-4–C-5–C-6 from the observed H-6 to C-8, C-2, and H-5 to C-7, three-bond correlations in the HMBC spectrum, as well as the observed H-6 $\beta$ /H-9 NOE. However, unlike in the case of **1**, another partial structure shown from the COSY spectrum of **2**, was that of  $\text{NCH}_2\text{CH}(\text{CH}_2)\text{CH}_2$ , instead of  $\text{NCH}_2\text{CH}(\text{CH}_2)\text{CH}_2\text{CHCH=}$  in **1**. This corresponds to the N-4–C-3–C-14(C-17)–C-15 fragment in **2** as deduced from the HMBC data ( $^3J$  from H-17 to C-3;

(14) (a) Flack, H. D. *Acta Crystallogr. A* **1983**, 39, 876–881. 1148. (b) Hooft, R. W. W.; Straver, L. H.; Spek, A. L. *J. Appl. Crystallogr.* **2008**, 41, 96–103.

(15) The crystals of **1** are monoclinic, belonging to space group  $P2_1$ , with  $a = 9.6564(3)\text{ \AA}$ ,  $b = 8.3932(2)\text{ \AA}$ ,  $c = 9.8414(3)\text{ \AA}$ ,  $\beta = 102.1833(3)^\circ$ ,  $V = 779.65(4)\text{ \AA}^3$ ,  $T = 100\text{ K}$ ,  $D_x = 1.322\text{ mg/m}^3$ , and  $Z = 2$ . The final  $R_1$  value is 0.0373 ( $wR_2 = 0.1064$ ) for 3040 reflections [ $I > 2\sigma(I)$ ]. Flack parameter [ $\chi = -0.17(19)$ ], Hooft parameter [ $y = -0.10(4)$ ]. CCDC no. 951763.

(16) HRESIMS found  $m/z$  293.1653 (calcd for  $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O} + \text{H}$ , 293.1648).

**Scheme 1.** Possible Biogenetic Pathway to **1** and **2**



H-3 to C-15; Figure 1). The other observed correlations (Figure 1) established the acetyl substituted, conjugated cyclohexene ring ( $^3J$  from H-17 to C-15, C-21; H-21 to C-15, C-19), the branching from N-4 to the quaternary C-16 (H-5 to C-3, C-16; H-3 to C-16), and the branching from the quaternary C-16 to C-2, C-17, and C-21 ( $^3J$  from H-21 to C-2; H-17 to C-21). The structure and relative configuration were also consistent with the NOE data (Figure 2). As for **1**, suitable crystals were obtained from chloroform solution and X-ray diffraction analysis confirmed the structure proposed based on the spectroscopic data, in addition to furnishing the absolute configuration (Figure 3).<sup>14,17</sup>

Although the basic ring systems of both the two new alkaloids have been encountered previously as intermediate

compounds in synthesis (for instance, the pentacyclic ring skeleton of **2** was assembled by Büchi in his synthesis of ibogamine by reaction of  $\beta$ -indolylacetyl chloride with the salt from a nicotinamide-derived ethyl 2-azabicyclo-[2.2.2]octan-6-one derivative, followed by treatment of the product with PTSA/AcOH,<sup>18</sup> while a pentacyclic ring system similar to **1**, but with different configuration and side-chain substitution, was obtained as a product from the oxidation of dehydroquebrachamine<sup>19</sup>), they are here isolated as optically active natural compounds for the first time, and represent two previously unencountered natural alkaloid skeletons.

A possible pathway to these alkaloids from an iboga precursor via a common cleavamine-type intermediate is shown in Scheme 1.<sup>20</sup> Oxidation of the keto-ibogamine **3** (which also occurs in the stem-bark extract) yields the  $\alpha$ -epoxide **4**, which on a lone pair-assisted Grob-like fragmentation results in cleavage of the C-16–C-21 bond, yielding an iminium ion of a cleavamine-type compound, **5**. Hydrolysis of this iminium ion **5** gives the enamine of the ketone–aldehyde **6**, which on an intramolecular enamine–aldehyde reaction gives the tetracyclic hydroxyindole-**9** **7**. Closure of **7** via path *a* (N-4 attack on imine carbon C-2, followed by dehydration) gives voatinggine (**1**), while the alternative closure via path *b* (deprotonation followed by N-4 attack on C-16, with concomitant aromatization and loss of OH) gives tabertinggine (**2**). Compounds **1** and **2** were tested for their cytotoxic effects against KB cells. While both compounds showed no significant effects toward both drug-sensitive as well as drug-resistant KB cells ( $IC_{50} > 25 \mu\text{g/mL}$ ); tabertinggine (**2**) however, showed moderate activity in reversing multidrug-resistance in vincristine-resistant KB (VJ300) cells ( $IC_{50} 13.3 \mu\text{g/mL}$  in the presence of  $0.1 \mu\text{g/mL}$  of vincristine).

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**Supporting Information Available.** Experimental procedures, 1D and 2D NMR, HRESIMS spectroscopic data, and X-ray crystallographic data (CIF) of **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

(17) The crystals of **2** are orthorhombic, belonging to space group  $P2_12_12_1$ , with  $a = 6.7462(2) \text{ \AA}$ ,  $b = 9.3007(3) \text{ \AA}$ ,  $c = 32.0517(9) \text{ \AA}$ ,  $V = 2011.06(11) \text{ \AA}^3$ ,  $T = 100 \text{ K}$ ,  $D_x = 1.419 \text{ mg/m}^3$ , and  $Z = 4$ . The final  $R_1$  value is 0.0588 ( $wR_2 = 0.1228$ ) for 3332 reflections [ $I > 2\sigma(I)$ ]. Flack parameter [ $x = 0.05(9)$ ], Hooft parameter [ $y = 0.005(16)$ ]. CCDC no. 951764.

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The authors declare no competing financial interest.