

## Norditerpenoid Alkaloids from *Delphinium nordhagenii*

Farzana Shaheen,<sup>\*,†</sup> Muhammad Zeeshan,<sup>†</sup> Manzoor Ahmad,<sup>†</sup> Shazia Anjum,<sup>†</sup> Shamshir Ali,<sup>†</sup> Hong-Kun Fun,<sup>§</sup> Muhammad Iqbal Choudhary,<sup>†,‡</sup> and Atta-ur-Rahman<sup>†,‡</sup>

H. E. J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical Sciences, University of Karachi, Karachi-75270, Pakistan, and X-Ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia

Received November 18, 2005

Three new norditerpenoid alkaloids, nordhagenine A (**1**), nordhagenine B (**2**), and nordhagenine C (**3**), along with a known alkaloid, lycocotinine, were isolated from the aerial parts of *Delphinium nordhagenii*. The structures of the new compounds **1** and **2** were also deduced on the basis of single-crystal X-ray diffraction studies.

*Delphinium* (Larkspur), an important genus of the family Ranunculaceae, is well known for its potential uses in medicine.<sup>1</sup> The genus is recognized as a rich source of biologically active and structurally complex diterpenoid and norditerpenoid alkaloids with febrifuge, sedative, cardiotonic, and analgesic activities.<sup>2–6</sup> *Delphinium nordhagenii* Wendelbo is a 15–30 cm high perennial herb found at an elevation of 2300–2800 m.<sup>7</sup> No phytochemical work has been reported on this plant. Previously, we have reported many diterpenoid and norditerpenoid alkaloids from *Aconitum* and *Delphinium* species.<sup>8–10</sup> In the present paper, we describe the isolation, characterization, and structure elucidation of three new lycocotinine-type norditerpenoid alkaloids, nordhagenine A (**1**), nordhagenine B (**2**), and nordhagenine C (**3**) along with a known compound, lycocotinine. The structures of compounds **1** and **2** were also deduced from single-crystal X-ray diffraction analysis.

Nordhagenine A (**1**) was obtained as a colorless crystalline compound and was assigned the molecular formula C<sub>25</sub>H<sub>37</sub>NO<sub>6</sub>, on the basis of EIMS (*m/z* = 449.1), X-ray diffraction, and NMR spectroscopic data. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of nordhagenine A (**1**) exhibited a close resemblance to that of the known compound bonvalotine,<sup>11</sup> except for the absence of an acetoxy group at C-6 and the presence of an *N*-ethyl group at δ<sub>H</sub> 1.01 (3H, t, *J* = 7.0 Hz), instead of an *N*-methyl group. The <sup>1</sup>H NMR spectrum exhibited three aliphatic *O*-methyl groups at δ<sub>H</sub> 3.28, 3.34, 3.41 (each 3H, s) and two singlets at δ<sub>H</sub> 4.98 and 4.86 for a methylenedioxy group (O–CH<sub>2</sub>–O). The IR spectrum showed characteristic signals at 3492 (OH groups) and 1083 (simple ether bonds), and the <sup>13</sup>C NMR spectrum also showed a signal at δ<sub>C</sub> 79.0 (C-5), indicating a hydroxyl group at this carbon. The long-range <sup>1</sup>H and <sup>13</sup>C NMR connectivities were established through HMBC correlations (Figure 1 and Table 1). Finally the structure and relative configuration of polycyclic diterpenoid alkaloid **1** were deduced on the basis of X-ray diffraction studies. The methoxy substituents at C-1, C-14, and C-16 are α-oriented. The hydroxyl group at C-5 is β-oriented (Figure 2).

Nordhagenine B (**2**) was obtained as a colorless crystalline compound and was assigned the molecular formula C<sub>26</sub>H<sub>39</sub>NO<sub>8</sub>, on the basis of EIMS (*M*<sup>+</sup> *m/z* = 493.2), X-ray diffraction, and NMR spectroscopic data. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of nordhagenine B (**2**) exhibited close resemblance to the known compound elatine,<sup>12</sup> with the difference in configuration at C-16: the hydroxyl group at C-16 was α-oriented in the new compound. In the <sup>1</sup>H NMR spectrum two aliphatic *O*-methyl groups resonated

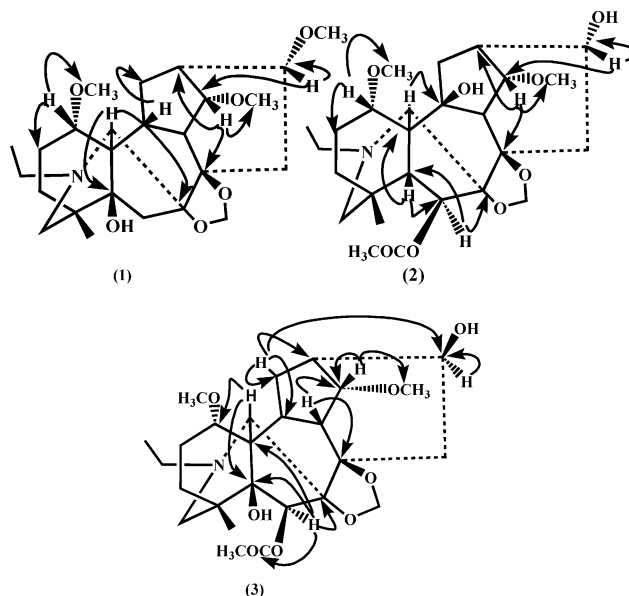


Figure 1. Key HMBC interactions in compounds **1**, **2**, and **3**.

Table 1. <sup>13</sup>C NMR Data of Compounds **1**, **2**, and **3** in CDCl<sub>3</sub>

C no.	<b>1</b>		<b>2</b>		<b>3</b>	
	<sup>13</sup> C	multiplicity	<sup>13</sup> C	multiplicity	<sup>13</sup> C	multiplicity
1	82.8	CH	79.0	CH	80.5	CH
2	21.0	CH <sub>2</sub>	26.3	CH <sub>2</sub>	26.2	CH <sub>2</sub>
3	28.2	CH <sub>2</sub>	38.0	CH <sub>2</sub>	28.4	CH <sub>2</sub>
4	39.8	C	34	C	34.0	C
5	79.0	C	50.5	C	77.0	C
6	34.0	CH <sub>2</sub>	77.2	CH	77.5	CH
7	84.1	C	92.7	C	92.5	C
8	83.9	C	81.8	C	83	C
9	42.02	CH	47.9	CH	40	CH
10	38.0	CH	80.2	CH	41.2	CH
11	54.8	C	50.32	C	55.0	C
12	27.4	CH <sub>2</sub>	37.2	CH <sub>2</sub>	38.9	CH <sub>2</sub>
13	40.0	CH	40.0	CH	44.9	CH
14	84.0	CH	83	CH	82.0	CH
15	42.2	CH <sub>2</sub>	36.05	CH <sub>2</sub>	39.1	CH <sub>2</sub>
16	83.8	CH	71.6	CH	71.4	CH
17	62.3	CH	64.2	CH	62.1	CH
18	21.0	CH <sub>3</sub>	21.6	CH <sub>3</sub>	21.4	CH <sub>3</sub>
19	61.6	CH <sub>2</sub>	56.7	CH <sub>2</sub>	60.8	CH <sub>2</sub>
20	51.5	N–Et	50.3	N–Et	51.8	N–Et
21	14.0		13.8		10.9	
22	94.5	CH <sub>3</sub>	93.8	CH <sub>2</sub>	95.1	CH <sub>2</sub>
23	56.2	OCH <sub>3</sub>	56.5	OCH <sub>3</sub>	58.0	OCH <sub>3</sub>
24	57.8	OCH <sub>3</sub>	57.6	OCH <sub>3</sub>	56.3	OCH <sub>3</sub>
25	58.8	OCH <sub>3</sub>	169.7	OAc	169.4	OAc
26			25.4		26.2	

at δ<sub>H</sub> 3.21 and 3.40 (each 2H, s). Two singlets at δ<sub>H</sub> 4.93 and 4.89 were assigned to a methylenedioxy group (O–CH<sub>2</sub>–O). The long-range <sup>1</sup>H and <sup>13</sup>C NMR connectivities were established through

\* To whom correspondence should be addressed. E-mail: farzanailias@yahoo.com. Tel: +92-21-4824930. Fax: +92-21-4819018, 19.

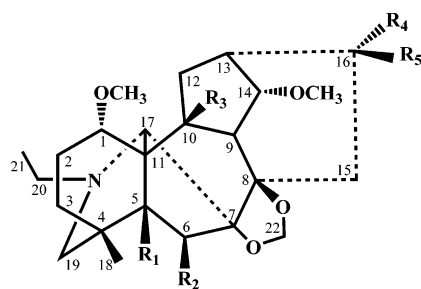
<sup>†</sup> H. E. J. Research Institute of Chemistry, University of Karachi.

<sup>‡</sup> Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi.

<sup>§</sup> Universiti Sains Malaysia.

HMBC correlations (Figure 1 and Table 1). The structure and relative configuration of polycyclic diterpenoidal alkaloid **2** were also unambiguously deduced on the basis of X-ray diffraction studies (Figure 2). The *O*-methyl substituents at C-1 and C-14 are  $\alpha$ -oriented. The acetoxy group at C-6 is  $\beta$ -oriented; the hydroxyl groups at C-10 and C-16 are  $\beta$ - and  $\alpha$ -oriented, respectively. The conformations of the rings in both of the alkaloids **1** and **2** are similar except for ring A (C1, C2, C3, C4, C5, and C11); ring A in alkaloid **2** is a twisted boat, while in **1** it is observed as a chair.

Nordhagenine C (**3**) was obtained as a colorless amorphous compound and was assigned the molecular formula  $C_{26}H_{39}NO_8$ , on the basis of EIMS ( $M^+$ ,  $m/z = 493$ ) and NMR spectroscopic data. The  $^1H$  and  $^{13}C$  NMR spectra of nordhagenine C (**3**) exhibited close resemblance with the known compound, bonvalotine,<sup>11</sup> except for the presence of the *N*-ethyl group at  $\delta_H$  1.02 (3H, t,  $J = 7.13$  Hz) and a hydroxyl group at position C-16. The two aliphatic *O*-methyl groups appeared at  $\delta_H$  3.23 and 3.50 (each 2H, s), and the two singlets appeared at  $\delta_H$  4.93 and 4.88 for a methylenedioxy group ( $O-CH_2-O$ ). The IR spectrum also showed absorbance at 3437 (OH groups) and 1086 (simple ether bonds), and the  $^{13}C$  NMR spectrum showed a signal at  $\delta_C$  71.4 (C-16), indicating a hydroxyl group at this carbon. The long-range  $^1H-^{13}C$  connectivities were established through HMBC correlations (Figure 1 and Table 1).



Nordhagenine A (**1**)  $R_1 = OH$ ,  $R_2, R_3, R_5 = H$   $R_4 = OCH_3$

Nordhagenine B (**2**)  $R_1, R_5 = H$ ,  $R_2 = OCOCH_3$ ,  $R_3 = R_4 = OH$

Nordhagenine C (**3**)  $R_1 = OH$ ,  $R_2 = OCOCH_3$ ,  $R_3, R_4 = H$ ,  $R_5 = OH$

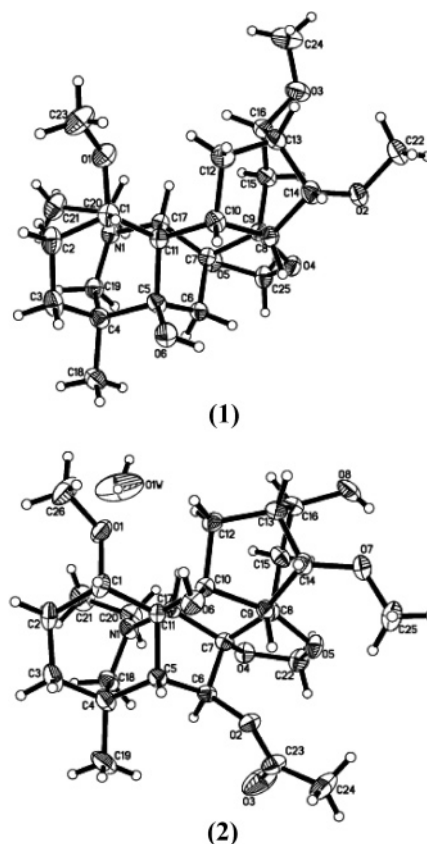
## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO DIP 360 polarimeter. IR spectra were recorded on a JASCO 302-A spectrophotometer. EIMS and HREIMS were recorded on JMS HX 110 with a data system and on JMS-DA 500 mass spectrometers. The  $^1H$  and  $^{13}C$  NMR spectra were recorded on Bruker NMR spectrometers operating at 600 and 400 MHz for  $^1H$  (150 and 100 MHz for  $^{13}C$ ). The chemical shift values are reported in ppm ( $\delta$ ) units, and the coupling constants ( $J$ ) are given in Hz.

**Chromatographic Conditions.** For TLC precoated aluminum sheets (silica gel 60F-254, E. Merck) were used. Visualization of the TLC plates was achieved under UV at 254 and 366 nm and by spraying with Dragendorff's reagent. Solvent system: *n*-hexane–acetone (8:2); 10 drops of  $Et_2NH$  were used.

**Plant Material.** The plant *D. nordhagenii* was collected from the village Kalam, Swat District, N. W. F. P. Pakistan, in August 2003. Botanical identification was done by Dr. Habib Ahmad, Jehanzeb Post Graduate College, Saidu Sharif, Swat. A voucher specimen (ND-09) was deposited in the herbarium of the Botany Department.

**Extraction and Isolation.** Dried and powdered aerial parts (4 kg) of the plant were extracted exhaustively with *n*-hexane ( $3 \times 8$  L) followed by 80% EtOH ( $3 \times 10$  L) at room temperature for 7 days (3 times). The filtrate was evaporated in vacuo to yield 460 g of residue. The residue was acidified to pH 2 by 5%  $H_2SO_4$  and extracted with  $CH_2Cl_2$  ( $3 \times 2$  L) to obtain a mixture (39.5 g). The acidic aqueous solution was basified (pH, 8–10) by using 10% KOH and extracted with  $CH_2Cl_2$  ( $5 \times 2$  L) to yield 6.5 g of crude mixed alkaloids. The crude alkaloidal mixture obtained was fractionated on a silica gel column (2.55 g), and five combined fractions were obtained. On repeated flash column chromatography using hexane–acetone– $Et_2NH$



**Figure 2.** X-ray structures of **1** and **2** showing the atom-numbering scheme.

(9:1:10 drops per 100 mL) nordhagenine A (**1**), nordhagenine B (**2**), and nordhagenine C (**3**) along with lycocotinine were obtained.

**Nordhagenine A (1):** plate-shaped colorless crystals (*n*-hexane–acetone (8:2), 5 mg); mp 178–180 °C;  $[\alpha]_D^{30} -3.20$  (*c* 0.4,  $CHCl_3$ );  $R_f = 0.48$ ; EIMS ( $m/z$ ) 449.1 ( $M^+$ ,  $C_{25}H_{37}NO_6$ ); IR  $\nu_{max}$   $CHCl_3$  3492 (OH), 1083 (simple ether bonds);  $^1H$  NMR (600 MHz,  $CDCl_3$ )  $\delta$  0.81 (3H, s, C-18), 1.04 (3H, t,  $J = 7.2$  Hz, *N*- $CH_2CH_3$ ), 3.22 (3H, s, OMe), 3.31 (3H, s, OMe), 3.41 (3H, s, OMe), 3.70 (1H, t,  $J = 4.59$  Hz, C-14), 4.85 and 4.98 (1H, each s,  $CH_2$ );  $^{13}C$  NMR (see Table 1).

**Nordhagenine B (2):** block-shaped colorless crystals (*n*-hexane–acetone (8:2), 10 mg); mp 200–203 °C;  $[\alpha]_D^{30} -3.70$  (*c* 0.4,  $CHCl_3$ );  $R_f = 0.38$ ; EIMS ( $m/z$ ) 493.2 ( $M^+$ ,  $C_{26}H_{39}NO_8$ ); IR  $\nu_{max}$   $CHCl_3$  3450 (OH), 1077 (simple ether bonds);  $^1H$  NMR (600 MHz,  $CDCl_3$ )  $\delta$  0.85 (3H, s, C-18), 1.05 (3H, t,  $J = 6.55$  Hz, *N*- $CH_2CH_3$ ), 2.15 (3H, s,  $OCOCH_3$ ), 3.23 (3H, s, OMe), 3.48 (3H, s, OMe), 3.66 (1H, t,  $J = 9.2$  Hz, C-16), 4.28 (1H, t,  $J = 5.1$  Hz, C-14), 4.7 and 4.8 (1H, each s,  $CH_2$ ), 5.5 (1H, s, C-6).  $^{13}C$  NMR (see Table 1).

**Nordhagenine C (3):** white amorphous compound (3 mg);  $[\alpha]_D^{30} -4.28$  (*c* 0.7,  $CHCl_3$ );  $R_f = 0.53$ ; EIMS ( $m/z$ ) 493.2 ( $M^+$ ,  $C_{26}H_{39}NO_8$ ); IR  $\nu_{max}$   $CHCl_3$  3437 (OH), 1086 (simple ether bonds);  $^1H$  NMR (600 MHz,  $CDCl_3$ )  $\delta$  0.84 (3H, s, C-18), 1.04 (3H, t,  $J = 7.1$  Hz, *N*- $CH_2CH_3$ ), 2.04 (3H, s, OAc), 3.23 (3H, s, OMe), 3.50 (3H, s, OMe), 3.66 (1H, t,  $J = 9.8$  Hz, C-16), 4.27 (1H, t,  $J = 4.9$  Hz, C-14), 4.8 and 4.9 (1H, each s,  $CH_2$ ), 5.4 (1H, s, C-6);  $^{13}C$  NMR (see Table 1).

**X-ray Data of Nordhagenine A (1).** A plate-shaped colorless crystal (*n*-hexane–acetone) of compound **1** with dimensions  $0.37 \times 0.12 \times 0.07$  mm was selected for X-ray diffraction studies.  $C_{25}H_{37}NO_6$ :  $M_f$ , 449.57; monoclinic;  $a = 9.4770(2)$  Å,  $b = 8.4071(2)$  Å,  $c = 14.4806(3)$  Å,  $V = 1145.97(4)$  Å<sup>3</sup>, space group =  $P2(1)$ ,  $Z = 2$ ,  $D_{calc} = 1.303$  mg/m<sup>3</sup>,  $F(000) = 488$ , Mo  $K\alpha$  ( $\lambda$  0.7107 Å). Intensity data of compound **1** were collected on a Bruker Smart APEX II, CCD 1-K area-detector diffractometer.<sup>13</sup> Data reductions were performed using SAINT. The structure was solved by direct methods<sup>14</sup> and refined by full-matrix least squares on  $F^2$  using the SHELXTL-PC package.<sup>15</sup> The intensity data within the  $\theta$  range 2.16–28.35° were collected at 100(2) K. A total of 22 841 reflections were recorded, of which 3055 reflections were observed on the basis of  $I > 2\sigma(I)$ . The final  $R$  and  $R_w$  were 0.0464 and 0.0958, respectively. The figure was plotted with the aid of ORTEP.<sup>16</sup>

**X-ray Data of Nordhagenine B (2).** A block-shaped colorless crystal (*n*-hexane–acetone) of compound **2** with dimensions 0.79 × 0.19 × 0.16 mm was selected for X-ray diffraction studies. C<sub>26</sub>H<sub>39</sub>NO<sub>8</sub>·0.7H<sub>2</sub>O (the water molecule is only 70.6% occupied in the unit cell): *M*<sub>r</sub> 506.29; orthorhombic; *a* = 12.3091(15) Å, *b* = 12.7389(15) Å, *c* = 16.375(2) Å, *V* = 2567.7(5) Å<sup>3</sup>, space group = *P*2(1)2(1)2(1), *Z* = 4, *D*<sub>calc</sub> = 1.310 mg/m<sup>3</sup>, *F*(000) = 1092, Mo Kα (*λ* 0.7107 Å). Intensity data of compound **2** was collected on a Bruker Smart APEX I, CCD 1-K area-detector diffractometer.<sup>17</sup> Data reductions were performed using SAINT. The structure was solved by direct methods<sup>14</sup> and refined by full-matrix least squares on *F*<sup>2</sup> using the SHELXTL-PC package.<sup>15</sup> The intensity data within the *θ* range 2.03–25.00° were collected at 293(2) K. A total of 13 502 reflections were recorded, of which 2566 reflections were observed on the basis of *I* > 2σ(*I*). The final *R* and *R*<sub>w</sub> were 0.0396 and 0.1045, respectively. The figure was plotted with the aid of PLUTO.<sup>16</sup>

Crystallographic data for compounds **1** and **2** have been deposited at Cambridge Crystallographic Data Center (CCDC 287321 and CCDC 287322), 12 Union Road, Cambridge, CB/EZ, UK (fax: 44-1223-336-033, e-mail: deposit@ccdc.cam.ac.uk).

**Acknowledgment.** We are grateful to Dr. H. Ahmad, plant taxonomist, Jahanzeb Post Graduate College, Saidu Sharif, Swat, NWFP, Pakistan, for help in the collection and identification of the plant, and we are also grateful to Islamic Development Bank for providing us financial support.

**Supporting Information Available:** This material is available free of charge via the Internet at <http://pubs.acs.org>.

#### References and Notes

- (1) Benn, M. H.; Jacyno, J. M. In *Alkaloids, Chemical and Biological Perspective*; Pelletier, S. W., Ed.; J. Wiley and Sons: New York, 1984; Vol. 2, p 153.
- (2) Reina, M.; Madinvavieta, A.; Gavin, J. A.; de la Fuente, G. *Phytochemistry* **1996**, *41*, 1235.
- (3) Pelletier, S. W.; Mody, N. V.; Joshi, B. S.; Schramma, L. C. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Wiley-Interscience: New York, 1984; Vol. 2, p 205.
- (4) Benn, M. H.; Jacyno, J. M. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; J. Wiley and Sons: New York, 1984; Vol. 1, p 153.
- (5) Yunusov, M. S. *Nat. Prod. Rep.* **1993**, *10*, 471.
- (6) Benn, M. H.; Jacyno, J. M. *The Toxicology and Pharmacology of Diterpenoid Alkaloids in Alkaloids: Chemical and Biological Perspectives*; J. Wiley and Sons: New York, 1983; Vol. 1, p 153.
- (7) Riedle, H.; Nasir, Y. J. *Flora of Pakistan*; Ali, S. I., Nasir, Y. J., Eds.; Times Press: Pakistan, 1991, Vol. 193, pp 30–31.
- (8) Atta-ur-Rahman; Fatima, N.; Akhtar, F.; Choudhary, M. I.; Khalid, A. New norditerpenoid alkaloids from *Aconitum falconeri*. *J. Nat. Prod.* **2000**, *63*, 1393.
- (9) Atta-ur-Rahman; Nasreen, A.; Akhtar, F.; Shekhani, M. S.; Clardy, J.; Parvez, M.; Choudhary, M. I. Antifungal diterpenoid alkaloids from *Delphinium denudatum*. *J. Nat. Prod.* **1997**, *60*, 472.
- (10) Shaheen, F.; Ahmad, M.; Khan, M. T. H.; Jalil, S.; Ejaz, A.; Sultankhodjaev, M. N.; Arfan, M.; Choudhary, M. I.; Atta-ur-Rahman. *Phytochemistry* **2005**, *66*, 935–940.
- (11) Jiang, Q. P.; Sung, W. L. *Heterocycles* **1984**, *22* (11), 2429–2432.
- (12) Pelletier, S. W.; Ross, S. A.; Kulanthaivel, P. *Tetrahedron* **1989**, *45* (7), 1887–1892.
- (13) APEX2 (Version 1.27), SAINT, and SADABS; Bruker AXS Inc.: Madison, WI, 2005.
- (14) Altomare, A.; Cascarano, M.; Giacovazzo, C.; Guagliardi, A. *J. Appl. Crystallogr.* **1993**, *26*, 343.
- (15) Sheldrick, G. M. *SHELXTL-PC* (Version 5.1); Siemens Analytical Instruments, Inc.: Madison, WI, 1997.
- (16) Johnson, C. K. *ORTEPII*; Report ORNL-5138; Oak Ridge National Laboratory: TN, 1976.
- (17) SMART and SAINT; Siemens Analytical X-Ray instruments Inc.: Madison, WI, 1969.

NP050478M