JAVACARBOLINE, A NEW β-CARBOLINE ALKALOID FROM THE STEM OF *PICRASMA JAVANICA* IN JAVA

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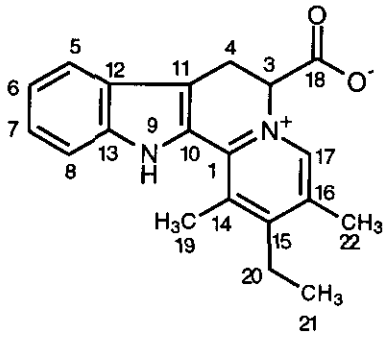
Abstract ---- Javacarboline, a new β-carboline alkaloid was isolated from the stem of *Picrasma javanica* (Simaroubaceae). Its structure was elucidated at the extensive ^{1}H and ^{13}C nmr studies involving ^{1}H-^{1}H COSY, HSQC and HMBC experiments and X-ray diffraction analysis.

During the alkaloidal investigation of Simaroubaceae plants,[1] *Picrasma javanica* BL. was studied. *P. javanica* is a medium-sized tree found in the New Guineas, Southeast Asia, and India. The decoctions of its bark are used in folk medicine as febrifuge and as a substitute for quinine.[2] From the chemical studies of the constituents of *P. javanica*, a number of quassinoids and alkaloids have been identified.[3,4] This paper describes the isolation and structural elucidation of a new β-carboline alkaloid, javacarboline (**1**) from the stem of *P. javanica*, which was collected in Java Island, Indonesia.

Compound (**1**) was obtained as pale-yellow prisms. The molecular formula, $C_{20}H_{20}N_2O_2$, was determined from its positive ion FAB-ms spectrum (*m/z* 321[M+H]$^+$) and from its ^{13}C nmr spectrum, 12 degrees of unsaturation was inferred. The ^{13}C and DEPT nmr spectra suggested the presence of three CH_3, two CH_2, one sp^3-CH, five sp^2-CH and nine sp^2-quaternary carbons. The HSQC[5] experiment allowed us to assign and correlate all the protons to corresponding carbons. The ir spectrum showed strong absorption due to the imino group (3422 cm^{-1}) and carboxylate ion group (1627, 1236 and 752 cm^{-1}). The uv absorbances (λ_{max} 235, 308, 380 nm) and ^{1}H nmr signals in the δ 7.2-7.7 region showed the presence of an indole system and



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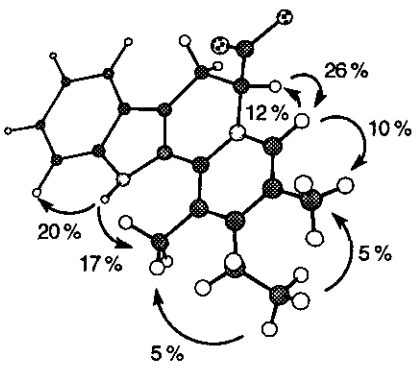


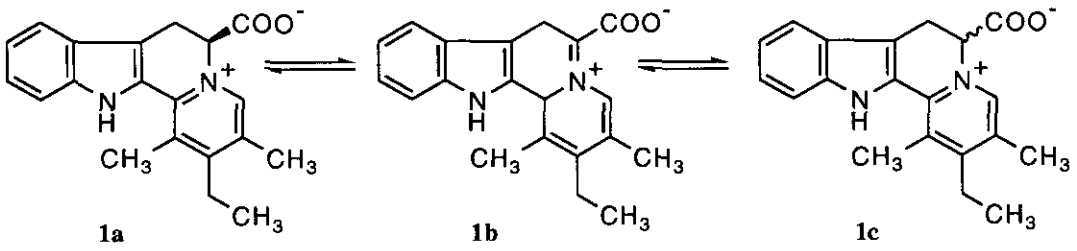
Figure 1. NOes (%) of **1**

an additional chromophore. The ^1H and ^{13}C nmr chemical shifts for the C-3 methine proton at δ 5.89 (1H, dd, *J*=6.1, 1.2 Hz) and the C-4 methylene protons at δ 3.55 (1H, dd, *J*=16.8, 6.1 Hz) and 3.84 (1H, dd, *J*=16.8, 1.2 Hz) indicated that the indole system partly consists of a trihydro-β-carboline moiety. The ^1H nmr spectrum identified seven separate spin systems; the protons on C-5 to C-8 were part of an indolic benzene ring and two three-proton singlets at δ 2.47 and 2.84 were assigned to aromatic methyl groups and methylene H-20 (δ 2.96, 2H, q, *J*=7.6 Hz) to methyl H-21 (δ 1.21, 3H, t, *J*=7.6 Hz) protons comprised an ethyl appendage. In addition, the ^1H and ^{13}C nmr spectra of **1** displayed signals for an isolated aromatic methine at C-17 (δ_H 8.84, δ_C 143.34) with a large $^1J_{CH}$ coupling constant (184 Hz), indicating a nitrogen atom adjacent to C-17. The signal for a D_2O-exchangeable proton was observed at δ 11.75 (1H, s, indolic NH). Furthermore, an HMBC[6] experiment (Table I) showed that the protonated aromatic carbon C-17 was correlated with H-3 and H-22, and the carboxylate ion group was attached to the C-3 position, whereas two methyls and the ethyl group were connected to quaternary carbons at C-14, C-16, and C-15, respectively. Difference nOe experiments provided further evidence for the structure of **1** shown in Figure 1. All these data indicate that compound (**1**) has the indolo[2,3-*a*]quinolizine system characteristic of plant alkaloids like flavopereirine[7] and sempervirine.[8] However, the positive ion FAB-ms of **1** (*m/z* 641 $[2M+H]^+$ and 321 $[M+H]^+$) suggested that it contained one molecule of dimeric β-carbolines. The ^{13}C nmr spectrum provided evidence for the presence of a symmetry element in the molecule, since only a limited number of signals, assigned as indicated in Table I, could be observed. To confirm this situation for compound (**1**), a single crystal X-ray diffraction study was carried out and compound (**1**) was clearly shown to have the racemic form. Figure 2 shows an ORTEP drawing of a *3S*-molecule (**1a**). The peak clearly observed near N1 on a

difference Fourier map was assigned to the hydrogen atom. The bond lengths of C18-O1=1.248(9) Å and C18-O2=1.231(8) Å indicated a zwitterionic structure of the molecule with a protonated indolic NH group and deprotonated carboxyl group. The asymmetric unit contained two molecules which form a dimeric system by means of two hydrogen bonds involving a hydrogen atom at N-1 of one molecule and the carboxylate ion oxygen atom at O-2 of the second molecule [distance N1-H···O2=2.864(8) Å] (Figure 3).

Racemization in this compound is clearly the result of **1a**→**1b** tautomerization (Scheme I), wherein the natural product (**1a**) is compromised by the biosynthetic pathway from *L*-tryptophan, and the enantiomer of **1a** can, therefore, be formed by the extraction procedure.

The new compound was tested for *in vitro* cytotoxicity in human tumor PC-6 cells and a murine lymphocytic leukemia P-388 cells and it was found cytotoxicity at GI_{50} 35.9 μg/ml and 32.5 μg/ml, respectively.



Scheme I

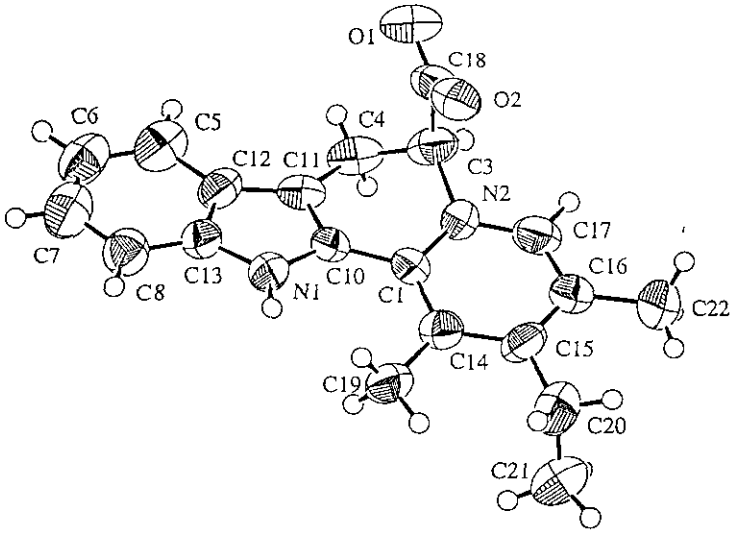


Figure 2. ORTEP drawing of 3*S*-molecule (**1a**).

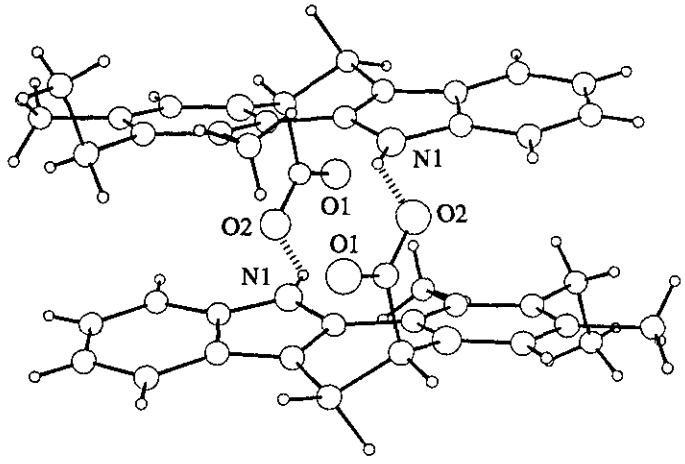


Figure 3. The dimer formed by the association of the two molecules of javacarboline (**1**), in the asymmetric unit. The dotted lines denote the hydrogen bonds.

Table I. ^{1}H and ^{13}C nmr data for **1** recorded in DMSO-d_6 at 353°K

C#	^{13}C[a]	$^{1}J_{CH}$ (Hz)[b]	^{1}H (ppm, J, Hz)[c]	HMBC[c] (C to H)
1	139.55			H3, H17, H19
3	67.17	146	5.89 dd (6.1, 1.2)	H4b, H17
4a	21.67	130	3.84 dd (16.8, 1.2)	
4b			3.55 dd (16.8, 6.1)	
5	119.70	160	7.74 br d (8.0)	H7
6	120.61	160	7.17 ddd (8.0, 7.1, 0.9)	H8
7	125.30	158	7.34 ddd (8.2, 7.1, 0.7)	H5
8	112.86	163	7.64 dt (8.2, 0.9)	H6
9(NH)			11.75*	
10	125.07			H4a, H9
11	115.71			
12	123.85			H5, H6, H9
13	139.10			H5, H7
14	130.52			H19, H20
15	162.03			H16, H17, H19, H20, H21, H22
16	131.91			H17, H20, H22
17	143.34	184	8.84 s	H3, H22
18	168.46			H3, H4a, H4b
19	15.52	128	2.84 s	
20	23.08	130	2.96 q (7.6)	H21
21	11.37	135	1.21 t (7.6)	
22	15.98	132	2.47 s	H17

[a](125 MHz); [b](100 MHz); [c](500 MHz); *(disappeared with D_2O).

EXPERIMENTAL

General experimental procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Ir spectrum was recorded on a JASCO 7300 FT-ir spectrometer. EI-ms and FAB-ms were measured using JEOL D-300 and DX-303 mass spectrometers, respectively. ^{1}H, ^{13}C and 2D nmr spectra were recorded using a JEOL A-500 (500 MHz for ^{1}H nmr and 125 MHz for ^{13}C nmr) spectrometer in dimethylsulfoxide-d_6, with TMS as the internal standard.

Isolation of javacarboline (1). The dried stem (3.7 kg) of *Picrasma javanica* was collected in Indonesia in July 1986. A voucher specimen has been deposited in the Department of Pharmacognosy, School of Pharmaceutical Sciences, Toho University. The stems were extracted with MeOH (49 l) at 60°C for 3 h. The extract was concentrated under reduced pressure to give a residue (922 g) and then an equal volume of H_2O was added. The aqueous solution was extracted with $CHCl_3$ (12 l) followed by *n*-BuOH (3.6 l). The $CHCl_3$-soluble fraction (73 g) gave javanicins N (30 mg),[4f] U (29 mg),[4g] V (28 mg),[4g] W (100 mg),[4g] neoquassin (10 mg),[4h] picrasin A (28 mg),[4h] and the *n*-BuOH fraction (70 g) gave javanicinosides E (3 mg),[4h] G (4 mg),[4h] I (60 mg),[4i] J (11 mg),[4i] K (4 mg),[4i] and L (2 mg)[4i] as reported in the previous papers. The remaining *n*-BuOH-soluble fraction was subjected to repeated column chromatography on silica gel (Merck) with $CHCl_3$-MeOH solvent gradient (5-100 % MeOH) and Diaion HP-20 (Mitsubishi Kasei) with H_2O-MeOH solvent gradient (20-100 % MeOH) and further purified by preparative medium-pressure liquid chromatography [column LiChroprep Rp-18 Merck, 20 mm i.d. x 300 mm, solvent system MeOH-H_2O (3:2), flow rate 0.6 ml/min, uv detector 254 nm and silica gel CQ-3 Fuji Gel, 10 mm i.d.x300 mm, solvent system $CHCl_3$-MeOH-H_2O (50:10:1), flow rate 0.4 ml/min, detector uv 254 nm] to afford javacarboline (**1**, 8 mg).

Javacarboline (1). Pale-yellow prisms (recrystallized from MeOH-H_2O), mp 220-222°. FT-ir ν_{max} (KBr) cm^{-1}: 3422, 2922, 1627, 1559, 1456, 1364, 1343, 1236, 1032, 752. Uv λ_{max} (MeOH) (log ε): 235 (4.14), 265 (sh, 3.87), 308 (3.94), 380 (4.02). Uv λ_{max}(MeOH+NaOH) (log ε): 235 (4.14), 265 (sh, 3.87), 308 (3.94), 380 (4.02). Uv λ_{max} (MeOH+HCl) (log ε): 235 (4.15), 250 (sh, 3.98), 308 (3.94), 380 (4.02). EI-ms *m/z* (rel. int.): 275 ($[M-CO_2H]^+$, 100), 261 (23), 246 (9), 218 (3). Positive ion FAB-ms *m/z*: 641 $[2M+H]^+$, 321 $[M+H]^+$, 275.

Crystal Data of 1. Crystallized from MeOH-H_2O (7:3) and belonging to triclinic space group $P\bar{1}$. Lattice constants and intensity data were measured on a Rigaku AFC-5R diffractometer equipped with a device for graphite-monochromated CuKα radiation. Crystal data: $C_{20}H_{20}N_2O_2 \cdot 4H_2O$, a= 10.859 (1) Å, b=13.470 (2) Å, c=7.9947 (9) Å, α=99.69(1)°, β=101.386 (10)°, γ=109.229 (10)°, V=1047.5(3) Å^3, Z=2, D_{calc}=1.244g/cm^3, μ(CuKα)=7.63 cm^{-1}. A total of 1188 independent reflections with I>3σ(Io) was used for structure analysis. Structure was resolved by a direct method (SIR88)[9] and expanded using Fourier techniques.[10] The structure was then refined by full-matrix least squares with anisotropic temperature factors for non-hydrogen and isotopic atoms for hydrogen atoms to an R factor of 0.068 (R_w=0.091), $(\Delta/\sigma)_{max}$=0.03, $\Delta\rho_{max}$=0.43, $\Delta\rho$=-0.16 eÅ^{-3}.

Table II. Atomic coordinates and isotropically equivalent thermal parameters with their standard deviation in parentheses for non-hydrogen atoms of javacarboline (**1a**)

Atom	x	y	z	Beq(Å^2)
O(1)	-0.0438(6)	0.1648(5)	0.0135(7)	7.2(2)
O(2)	-0.1793(5)	0.0383(4)	0.1133(7)	5.6(1)
N(1)	-0.5727(5)	0.0169(4)	-0.2146(7)	4.4(2)
N(2)	-0.3083(6)	-0.0976(5)	-0.2052(7)	4.7(2)
C(1)	-0.4377(7)	-0.1043(6)	-0.2085(8)	4.1(2)
C(3)	-0.1991(7)	0.0063(6)	-0.197(1)	5.2(2)
C(4)	-0.2508(8)	0.0652(6)	-0.3173(10)	5.5(2)
C(5)	-0.3987(9)	0.2497(7)	-0.3514(10)	6.2(2)
C(6)	-0.490(1)	0.3035(6)	-0.358(1)	6.8(3)
C(7)	-0.610(1)	0.2616(8)	-0.317(1)	7.0(3)
C(8)	-0.6487(8)	0.1659(7)	-0.2642(10)	5.8(2)
C(10)	-0.4612(6)	-0.0082(5)	-0.2324(8)	3.9(2)
C(11)	-0.3756(7)	0.0739(6)	-0.2852(9)	4.5(2)
C(12)	-0.4374(8)	0.1506(6)	-0.3027(9)	4.7(2)
C(13)	-0.5596(8)	0.1120(6)	-0.2574(8)	4.4(2)
C(14)	-0.5311(7)	-0.2018(6)	-0.1974(8)	4.5(2)
C(15)	-0.4876(8)	-0.2884(5)	-0.1732(10)	5.3(2)
C(16)	-0.3561(8)	-0.2781(6)	-0.1720(9)	5.2(2)
C(17)	-0.2732(7)	-0.1831(7)	-0.1890(10)	5.6(2)
C(18)	-0.1357(8)	0.0757(7)	-0.003(1)	5.1(2)
C(19)	-0.6734(7)	-0.2133(6)	-0.210(1)	5.7(2)
C(20)	-0.5872(9)	-0.3897(7)	-0.149(1)	6.9(3)
C(21)	-0.6690(9)	-0.4716(7)	-0.319(1)	8.0(3)
C(22)	-0.3045(9)	-0.3678(7)	-0.151(1)	8.4(3)

Table III. Bond length (Å) with their standard deviations in parentheses for javacarboline (**1a**)

Bond	Length	Bond	Length	Bond	Length
O(1) - C(18)	1.248(9)	C(3) - C(4)	1.48(1)	C(11)- C(12)	1.416(9)
O(2) - C(18)	1.231(8)	C(3) - C(18)	1.557(10)	C(12)- C(13)	1.400(9)
N(1) - C(10)	1.386(8)	C(4) - C(11)	1.466(10)	C(14)- C(15)	1.422(9)
N(1) - C(13)	1.351(8)	C(5) - C(6)	1.40(1)	C(14)- C(19)	1.483(9)
N(2) - C(1)	1.372(8)	C(5) - C(12)	1.408(10)	C(15)- C(16)	1.387(9)
N(2) - C(3)	1.488(8)	C(6) - C(7)	1.37(1)	C(15)- C(20)	1.51(1)
N(2) - C(17)	1.346(9)	C(7) - C(8)	1.38(1)	C(16)- C(17)	1.346(10)
C(1) - C(10)	1.435(9)	C(8) - C(13)	1.386(9)	C(16)- C(22)	1.51(1)
C(1) - C(14)	1.400(9)	C(10)- C(11)	1.378(9)	C(20)- C(21)	1.50(1)

Table IV. Bond angles (degree) with their standard deviation in parentheses for javacarboline (**1a**)

Bond	Angle	Bond	Angle	Bond	Angle
N(2) - C(1) - C(10)	113.6(6)	C(4) - C(11)- C(10)	119.0(7)	C(16)- C(15)- C(20)	121.6(7)
N(2) - C(1) - C(14)	118.5(6)	C(4) - C(11)- C(12)	133.8(7)	C(15)- C(16)- C(17)	117.3(7)
C(10)- C(1) - C(14)	127.8(6)	C(10)- C(11)- C(12)	107.1(6)	C(15)- C(16)- C(22)	122.5(7)
N(2) - C(3) - C(4)	110.7(6)	C(5) - C(12)- C(11)	133.2(8)	C(17)- C(16)- C(22)	120.1(8)
N(2) - C(3) - C(18)	110.0(6)	C(5) - C(12)- C(13)	119.9(7)	N(2) - C(17)- C(16)	124.8(7)
C(4) - C(3) - C(18)	112.8(6)	C(11)- C(12)- C(13)	106.9(6)	O(1) - C(18)- O(2)	128.1(7)
C(3) - C(4) - C(11)	109.9(6)	N(1) - C(13)- C(8)	128.9(7)	O(1) - C(18)- C(3)	113.5(8)
C(6) - C(5) - C(12)	116.9(8)	N(1) - C(13)- C(12)	108.5(6)	O(2) - C(18)- C(3)	118.4(7)
C(5) - C(6) - C(7)	121.3(8)	C(8) - C(13)- C(12)	122.6(8)	C(15)- C(20)- C(21)	112.9(7)
C(6) - C(7) - C(8)	122.8(8)	C(1) - C(14)- C(15)	119.3(6)	C(10)- N(1) - C(13)	109.0(5)
C(7) - C(8) - C(13)	116.4(8)	C(1) - C(14)- C(19)	119.7(7)	C(1) - N(2) - C(3)	121.9(6)
N(1) - C(10)- C(1)	127.3(6)	C(15)- C(14)- C(19)	120.9(7)	C(1) - N(2) - C(17)	120.0(6)
N(1) - C(10)- C(11)	108.4(6)	C(14)- C(15)- C(16)	119.9(6)	C(3) - N(2) - C(17)	117.7(6)
C(1) - C(10)- C(11)	124.2(6)	C(14)- C(15)- C(20)	118.5(7)		

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REFERENCES AND NOTES

1. H.-Y., Li, K. Koike, and T. Ohmoto, *Chem. Pharm. Bull.*, 1993, **41**, 1807 and references cited therein; T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, 1984, **32**, 170 and references cited therein; K. Mitsunaga, K. Koike, T. Tanaka, Y. Ohkawa, Y. Kobayashi, T. Sawaguchi, and T. Ohmoto, *Phytochemistry*, 1994, **35**, 799.

2. T. C. White, "*Tree Flora of Malaya*", Longman, 1973, Vol.II, p. 351; N. H. Ridely, "*The Flora of the Malay Peninsula*", A. Sher, Amsterdam, 1976, Vol. I, p. 361.

3. S. R. Johns, J. A. Lamberton, and A. A. Sioumis, *Aust. J. Chem.*, 1970, **23**, 629; T. Ohmoto, K. Koike,and K. Kagei, *Shoyakugaku Zasshi*, 1987, **41**, 338.

4. a) T. Ohmoto, K. Koike, K. Mitsunaga, H. Fukuda, K. Kagei, T. Kawai, and T. Sato, *Chem. Pharm. Bull.*, 1989, **37**, 993; b) T. Ohmoto, K. Koike, K. Mitsunaga, H. Fukuda, and K. Kagei, *Chem. Pharm. Bull.*, 1989, **37**, 2991; c) K. Koike and T. Ohmoto, *Phytochemistry*, 1990, **29**, 2617; K. Koike, K. Mitsunaga, and T. Ohmoto, *Chem. Pharm. Bull.*, 1990, **38**, 2746; d) K. Koike, K. Ishii, K. Mitsunaga, and T. Ohmoto, *Phytochemistry*, 1991, **30**, 933; e) K. Koike, K. Ishii, K. Mitsunaga, and T. Ohmoto, *J. Nat. Prod.*, 1991, **54**, 837; f) K. Koike, K. Ishii, K. Mitsunaga, and T. Ohmoto, *Chem. Pharm. Bull.*, 1991, **39**, 939; g) K. Koike, K. Ishii, K. Mitsunaga, and T. Ohmoto, *Chem. Pharm. Bull.*, 1991, **39**, 2021; h) K. Ishii, K. Koike, and T. Ohmoto, *Phytochemistry*, 1991, **30**, 4099; i) K. Koike and T. Ohmoto, *J. Nat. Prod.*, 1992, **55**, 482; j) M. Yoshikawa, E. Harada (née) Uchida, S. Aoki, J. Yamahara, N. Murakami, H. Shibuya, and I. Kitagawa, *Chem. Pharm. Bull.*, 1993, **41**, 2101.

5. G. Otting and K. Wüthrich, *J. Magn. Resonance*, 1988, **76**, 569.

6. A. Bax, *J. Am. Chem. Soc.* 1986, **108**, 2093.

7. E. Bächli, C. Vamvacas, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, 1957, **40**, 1167.

8. V. Prelog, *Helv. Chim. Acta*, 1948, **31**, 588.

9. M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, G. Polidori, R. Spagna, and D. Viterbo, *J. Appl. Cryst.*,1989, **22**, 389.

10. DIRDIF92: P. T. Beurskens, G. Admiraal, G. Beurskens, W. P. Bosman, S. Garcia-Granda, R. O. Gould, J. M. M. Smits, and C. Smykalla, The DIRDIF program system, Technical Report of the Crystallography Laboratory, University of Nijmegen, The Netherlands, 1992.

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