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MILLAURINE AND ACETYLMILLAURINE: ALKALOIDS FROM *MILLETTIA LAURENTII*[1]

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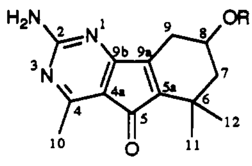
ABSTRACT.—Two guanidine alkaloids with a new skeleton, millaurine [**1**] and *O*-acetylmillaurine [**2**], have been isolated from the seeds of *Millettia laurentii* (Leguminosae). Their structures were determined from spectral studies including ir, ms, and nmr and X-ray crystallography.

The genus *Millettia* (Leguminosae) is represented by over 200 species and is distributed in tropical Africa, Asia, and Australasia (1). The seeds and other parts of *Millettia* species have been shown to have insecticidal (2) and piscicidal (3) activities due to the presence of such flavonoids as rotenone or related compounds; they are also used in folk medicine (4). Chemical studies on this genus have yielded a variety of flavonoids, especially prenylated isoflavones. In Cameroon, various parts of *Millettia* plants are used as insecticides and piscicides, as agents for the destruction of worms and snails, and as a cure for intestinal parasites. These uses, coupled with the abundance of *Millettia* species in Cameroon (5), prompted us to investigate the phytochemistry of *Millettia* as part of an on-going study (6,7) on Cameroonian medicinal plants. In this paper, we report the isolation, from the seeds of *Millettia laurentii* De Wild., and the structure elucidation of two alkaloids with a new skeleton, millaurine [**1**] and *O*-acetylmillaurine [**2**].

The ground and defatted seeds of *M. laurentii* were extracted with EtOAc. Si gel chromatography of this extract using gradient elution from C_6H_6 to EtOAc yielded acetylmillaurine [**2**] (40% EtOAc) and millaurine [**1**] (100% EtOAc), which were further crystallized from EtOH and MeOH, respectively.

The molecular formula $C_{14}H_{17}N_3O_2$ for millaurine [**1**] was determined by ^{13}C-nmr (Table 1) and hreims data. The presence of signals for seven sp^2 hybridized carbon atoms, among which was a carbonyl carbon of a conjugated ketone, indicated that the compound must be tricyclic. The ^{1}H-nmr spectrum (Table 1) was deceptively simple, showing only signals for a methinoxy, two methylene, and three Me groups. The ^{1}H-^{1}H COSY indicated the methinoxy proton to be vicinal to the two methylenes (C-7 and C-9); its large coupling constant values with the protons at δ_H 1.62 (7ax) and 2.27 (9ax) indicated that they were all axial. This point was confirmed by the observation of a W coupling



1 R=H
2 R=Ac

[1]Part 1 in the series "The *Millettia* of Cameroon."

TABLE 1. ^{1}H- and ^{13}C-nmr Data for Millaurine [**1**] and *O*-Acetylmillaurine [**2**] ($CDCl_3$).

Position	Compound						
	1				**2**		
	δ_C	δ_H	*J* (Hz)	HMBC ^{13}C with ^{1}H:	δ_C	δ_H	*J* (Hz)
2	164.1 s	—		—	162.3 s	—	
4	162.2 s	—		10	162.3 s	—	
4a	111.0 s	—		10	110.8 s	—	
5	192.7 s	—		9ax	192.5 s	—	
5a	148.1 s	—		7ax,7eq,9ax,9eq,11,12	146.8 s	—	
6	33.7 s	—		7ax,7eq,9ax,11,12	33.1 s	—	
7 ax	48.4 t	1.62 dd	12.8,11.2	9ax,9eq,11,12	43.9 t	1.73 dd	13.1,10.4
eq		1.83 ddd	12.8,3.4,1.7			1.82 ddd	13.1,3.2,1.1
8 ax	65.3 d	4.12 dddd	11.2,8.9, 5.3,3.4	7ax,7eq,9ax,9eq	67.6 d	5.15 dddd	10.4,7.9, 5.5,3.2
9 ax	30.9 t	2.27 dd	18.4,8.9	7ax,7eq	27.5 t	2.39 dd	18.8,7.9
eq		2.92 ddd	18.4,5.3,1.7			2.94 ddd	18.8,5.5,1.1
9a	147.7 s	—			147.8 s	—	
9b	176.9 s	—		9ax,9eq	176.6 s	—	
10	19.9 q	2.43 s			19.9 q	2.43 s	
11	28.0 q	1.36 s		7ax,7eq,9ax,9eq	27.8 q	1.35 s	
12	28.0 q	1.26 s		7ax,7eq,9ax,9eq	27.9 q	1.29 s	
NH_2	—	5.56 s			—	5.78 s	
OAc: CO-					170.4 s	—	
Me-					21.2 q	2.05 s	

(^{4}J=1.7 Hz) between their respective geminal protons at δ_H 1.83 (7eq) and 2.92 (9eq) that were equatorial. The OH group is thus equatorial.

The HMBC spectrum gave information allowing the determination of the structure of the hydroxydimethylcyclohexene moiety; long range correlations were observed between the carbon atoms at δ_C 148.1 (C-5a), 33.7 (C-6) and 48.4 (C-7) and the Me protons at δ_H 1.26, and 1.36, between C-5a, C-6, C-8, and C-9, and the methylene protons at δ_H 1.83 (7eq) and 1.62 (7ax) and between C-5a, C-7, and C-8 and the methylene protons at δ_H 2.92 (9eq) and 2.27 (9ax). The carbon atom at δ_C 33.7 (C-6) gave a ^{4}J correlation with this latter proton 9ax.

The carbonyl carbon at δ_C 192.7 (C-5) also showed a correlation to H-9ax, whereas the carbon atom at δ_C 176.9 showed cross peaks to both H-9ax and H-9eq suggested to be involved in a cyclopentadienone ring. The carbon signal at δ_C 164.1 showed no correlation in the HMBC, and the carbon atoms at δ_C 162.2 (C-4) and 111.0 (C-4a) were correlated only with the Me protons at δ_H 2.43 (C-10). These deficient data prevented further assembling of the partial structures.

Finally, the structure of millaurine [**1**] was established from X-ray analysis of a single crystal. Crystals were obtained by slow crystallization of **1** in CH_2Cl_2/MeOH. Figure 1 shows the structure of **1**; the three nitrogen atoms are linked to the same carbon atom forming a guanidine moiety with two of them being involved in a pyrimidine ring. The final atomic coordinates are given in Table 2. As deduced from nmr measurements, the OH group appeared to be equatorial. The asymmetric unit contained two molecules which form a planar dimeric system by means of two hydrogen bonds involving an hydrogen atom at N-10 of one molecule and the heterocyclic nitrogen atom N-1′ of the second molecule [distance $N10\text{-}H_a\cdots N1'$=3.121 (12) Å, angle 172.9 (4)°] and reciprocally a hydrogen atom at N-10′ and the nitrogen atom N-1, [$N10'$-H·····$N1$=3.015 (8) Å, angle 172.5 (2)°]. In the crystal packing of the molecules (Figure 2) two other hydrogen bonds were observed, formed between the OH groups O15-H or O15′-H and the nitrogen atom N-3′ or N-3, linking together the different dimers around the twofold axes [$O15$-H·····$N3'$=2.888 (6) Å, angle 163.5 (5)°] and [$O15'$-H·····$N3$=2.862 (5) Å,

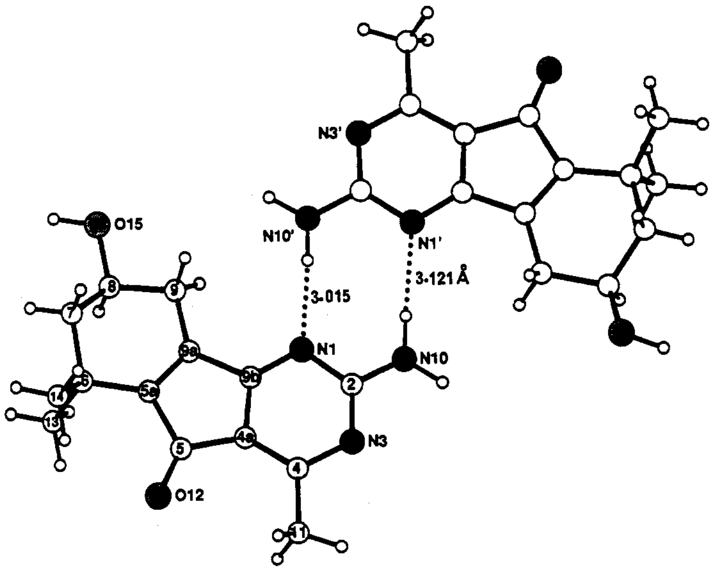


FIGURE 1. The planar dimer formed by the association of the two molecules of millaurine [**1**], in the asymmetric unit.

angle 155.7 (2)°]. In addition, one molecule of CH_2Cl_2 was found on the binary axis crystallizing for two molecules of millaurine.

The second isolated compound, **2**, was identified as *O*-acetylmillaurine. The hreims data indicated the molecular formula $C_{16}H_{19}N_3O_3$ and showed a molecular ion 42 mass units higher than that of **1**. The ^{1}H- and ^{13}C-nmr spectra (Table 1) were similar to those of **1**, differing mainly by the presence of an acetyl group and by a shift of the methinoxy proton to δ_H 5.15. The ^{1}H- and ^{13}C-nmr spectral assignments arose from analysis of the HMQC and HMBC spectra. The cross peaks depicted in the HMBC spectrum were similar to those previously observed in the HMBC spectrum of **1** and led to the same partial structures.

The final structure **2** of acetylmillaurine was established from the X-ray analysis of a single crystal obtained by slow crystallization of acetylmillaurine in EtOH. The final atomic coordinates are given in Table 3. The acetylmillaurine crystal packing is characterized by infinite chains of molecules parallel to the *a* axis direction. Each molecule (x,y,z) is hydrogen-bonded to two molecules of symmetry $(\pm 0.5+x, 0.5-y, -z)$ through the hydrogen atoms H_a and H_b of its nitrogen atom N-10 [distance $N10$-H_a·····$N1$=3.00 (1) Å, angle 152.1°; $N10$-H_b·····$N3$=3.10 (1) Å, angle 163.1°] as seen in Figure 3. In contrast to millaurine, the moieties of each dimeric association were not coplanar: the dihedral angle between the mean plane of each molecule was 65.5°.

Ovalin, an amino acid related to pipecolic acid, was previously isolated from *Millettia ovalifolia* (8). Millaurine [**1**] and its naturally occurring *O*-acetyl derivative **2** are the first characterized alkaloids from *Millettia* species and have a new type of three-ring skeleton, with a guanidine and a terpenic moiety.

EXPERIMENTAL

GENERAL METHODS.—^{1}H- (300.13 MHz) and ^{13}C- (75.47 MHz) nmr spectra were performed on an AC 300 Bruker spectrometer. Eims and cims spectra were obtained with a Nermag Sidar V 3.0 mass spectrometer and the hrms with a V.G. Analytical ZAB-HF mass spectrometer.

PLANT MATERIAL.—Seeds of *M. laurentii* were collected in Yaounde, Cameroon, in May 1990. Voucher specimens identifying the sample are on deposit in the National Herbarium, Yaounde.

TABLE 2. Final Fractional Coordinates ($\times 10^4$) for Millaurine [**1**] and the Equivalent Isotropic Thermal Factor U_{eq} ($Å^2 \times 10^3$).

Position	x	y	z	U_{eq}[a]
N-1	2944 (2)	4079 (13)	3156 (3)	51 (8)
C-2	2919 (3)	3603 (14)	2477 (4)	52 (10)
N-3	3181 (2)	2121 (13)	2446 (3)	58 (10)
C-4	3523 (3)	969 (14)	3159 (4)	54 (11)
C-4a	3580 (3)	1333 (14)	3879 (4)	52 (11)
C-5	3921 (3)	515 (16)	4759 (4)	63 (12)
C-5a	3801 (3)	1745 (15)	5232 (4)	54 (10)
C-6	4043 (3)	1469 (14)	6151 (4)	53 (10)
C-7	3885 (3)	3187 (14)	6398 (3)	60 (10)
C-8	3208 (3)	3962 (13)	5649 (4)	55 (10)
C-9	3138 (3)	4514 (1)	4867 (4)	60 (11)
C-9a	3411 (3)	3093 (13)	4676 (3)	46 (10)
C-9b	3284 (3)	2938 (14)	3838 (4)	48 (10)
N-10	2578 (3)	4696 (13)	1777 (3)	70 (10)
C-11	3803 (4)	−707 (17)	3104 (5)	85 (14)
O-12	4225 (3)	−911 (13)	5042 (4)	104 (12)
C-13	3701 (4)	−196 (15)	6136 (5)	107 (19)
C-14	4783 (3)	1185 (19)	6837 (4)	88 (14)
O-15	3084 (2)	5547 (13)	5913 (3)	91 (10)
CL-1	4738 (2)	4626 (12)	4096 (2)	204 (10)
C-20	5000	5871 (24)	5000	147 (40)
N-1′	2041 (2)	8372 (13)	1818 (3)	53 (8)
C-2′	2073 (3)	8755 (14)	2511 (4)	56 (11)
N-3′	1804 (2)	10215 (13)	2563 (3)	59 (10)
C-4′	1479 (3)	11393 (14)	1888 (4)	60 (12)
C-4a′	1431 (3)	11109 (13)	1169 (3)	50 (11)
C-5′	1113 (3)	12124 (14)	328 (4)	65 (13)
C-5a′	1277 (3)	11068 (14)	−140 (4)	54 (11)
C-6′	1081 (3)	11504 (14)	−1016 (4)	48 (10)
C-7′	1510 (3)	10344 (13)	−1087 (4)	55 (11)
C-8′	1584 (3)	8387 (14)	−830 (4)	49 (10)
C-9′	1861 (3)	8183 (11)	122 (4)	63 (11)
C-9a′	1629 (3)	9572 (13)	363 (3)	45 (10)
C-9b′	1722 (3)	9587 (14)	1178 (3)	43 (9)
N-10′	2401 (3)	7579 (14)	3193 (3)	89 (13)
C-11′	1178 (4)	12988 (17)	1950 (5)	91 (16)
O-12′	780 (3)	13464 (13)	47 (3)	102 (12)
C-13′	351 (3)	11122 (18)	−1784 (4)	81 (13)
C-14′	1216 (4)	13496 (14)	−1042 (4)	78 (13)
O-15′	2027 (2)	7441 (13)	−856 (3)	70 (8)
CL-2	182 (2)	7925 (12)	858 (2)	194 (9)
C-20′	0	6709 (23)	0	132 (35)

[a]$U_{eq} = 1/3\ \Sigma_i \Sigma_j\ U_{ij}\ a_i^*\ a_j^*\ a_i \cdot a_j$.

ISOLATION OF COMPOUNDS.—Ground seeds (28 kg) were defatted with hexane and then extracted with EtOAc to give 900 g of material. An aliquot portion (300 g) of this material was subjected to cc over Si gel. Elution with C_6H_6 followed by C_6H_6 containing increasing amounts of EtOAc gave, from 40% EtOAc, a fraction containing one major compound. This fraction was dissolved in hot EtOH and left to stand at room temperature. A yellow compound precipitated, was filtered, and was washed with cold EtOH followed by Et_2O to give acetylmillaurine [**2**] (120 mg).

One of the fractions eluted with 100% EtOAc had crystals suspended in an oil. It was dissolved in hot Me_2CO, from which a solid precipitated. This solid was washed repeatedly with cold EtOH, dissolved in hot MeOH, and left to stand at room temperature. Yellow crystals, millaurine [**1**], precipitated (700 mg).

Millaurine [**1**].—Yellow needles: mp 248–249° (MeOH); $[\alpha]^{21}D$ +20.6° (c=0.18, MeOH); ir (KBr)

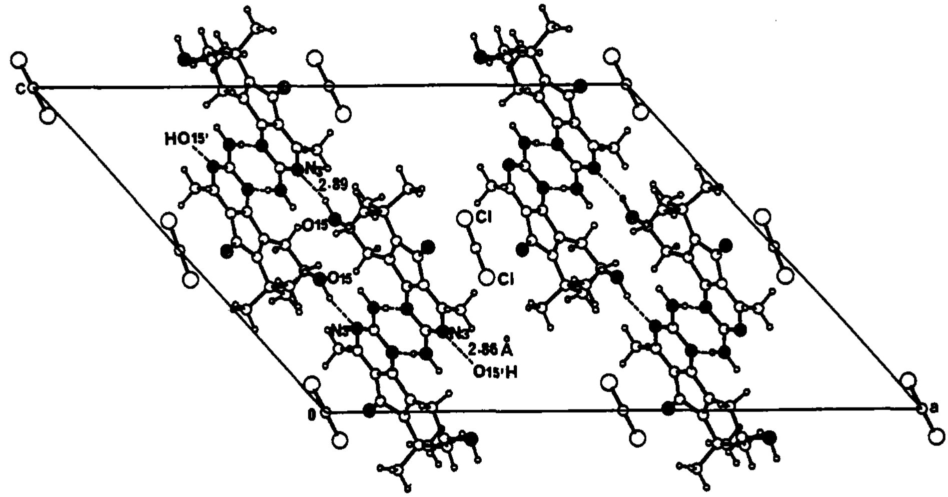


FIGURE 2. The molecular crystal packing of millaurine projected down the b axis.

ν max (cm^{-1}) 3466, 3343, 3223, 2962, 2941, 1712, 1706, 1633, 1598, 1474, 1402, 1363, 1324, 1255, 1202, 1174, 1056, 1030, 1014, 865, 807; eims *m/z* (rel. int.) 260 (21), $[M]^+$ 259 (100), 244 (37), 240 (24), 230 (31), 226 (65), 216 (35), 212 (29), 198 (23), 188 (22), 146 (12), 130 (14), 115 (15), 91 (22), 77 (34), 67 (43); cims (NH_3) *m/z* $[M+H]^+$ 260; hrms *m/z* $[M]^+$ 259.1327 (calcd for $C_{14}H_{17}N_3O_2$, 259.1321).

O-*Acetylmillaurine* [**2**].—Yellow needles: mp 218–219° (EtOH); $[\alpha]^{21}D$ +51.3° (c=0.34, MeOH); ir (KBr) ν max (cm^{-1}) 3336, 3194, 2951, 1740, 1700, 1656, 1629, 1591, 1557, 1495, 1436, 1395, 1239, 1036, 882, 860, 812; eims *m/z* (rel. int.) $[M]^+$ 301 (0.5), 270 (1), 241 (57), 240 (36), 226 (100), 212 (16), 198 (13), 184 (7), 143 (8), 115 (4), 67 (8); cims (NH_3) *m/z* (rel. int.) $[M+H]^+$ 302 (100), 241 (49), 226 (48); hrms *m/z* $[M+H]^+$ 302.1516 (calcd for $C_{16}H_{20}N_3O_3$, 302.1505).

TABLE 3. Final Fractional Coordinates ($\times 10^4$) for Acetylmillaurine [**2**] and the Equivalent Thermal Factor U_{eq} ($Å^2 \times 10^3$).

Position	x	y	z	U_{eq}
N-1	−4348 (10)	1923 (5)	1017 (4)	30 (7)
C-2	−5826 (12)	1853 (6)	489 (5)	31 (8)
N-3	−7287 (10)	1175 (6)	526 (4)	37 (7)
C-4	−7301 (13)	520 (6)	1138 (6)	39 (9)
C-4a	−5886 (12)	533 (6)	1707 (5)	31 (8)
C-5	−5437 (13)	−50 (7)	2424 (5)	33 (8)
C-5a	−3568 (13)	402 (6)	2746 (5)	31 (8)
C-6	−2456 (14)	15 (8)	3439 (5)	37 (8)
C-7	−519 (13)	556 (7)	3446 (5)	40 (9)
C-8	−640 (12)	1680 (7)	3228 (6)	41 (9)
C-9	−1396 (13)	1854 (7)	2411 (6)	40 (9)
C-9a	−3063 (11)	1179 (6)	2259 (5)	30 (8)
C-9b	−4464 (11)	1270 (6)	1616 (5)	30 (8)
N-10	−5824 (10)	2511 (6)	−106 (4)	36 (7)
C-11	−8863 (15)	−271 (8)	1132 (7)	60 (11)
O-12	−6338 (9)	−745 (4)	2728 (4)	48 (6)
C-13	−3540 (16)	223 (7)	4204 (5)	49 (10)
C-14	−2116 (14)	−1146 (8)	3361 (6)	54 (11)
O-15	1267 (9)	2140 (5)	3236 (4)	45 (6)
C-16	1949 (15)	2455 (8)	3931 (6)	52 (12)
O-17	1157 (13)	2344 (7)	4554 (4)	90 (11)
C-18	3828 (16)	2977 (9)	3828 (7)	67 (13)

[a] $U_{eq} = 1/3 \Sigma_i \Sigma_j U_{ij} a_i^* a_j^* a_i \cdot a_j$.

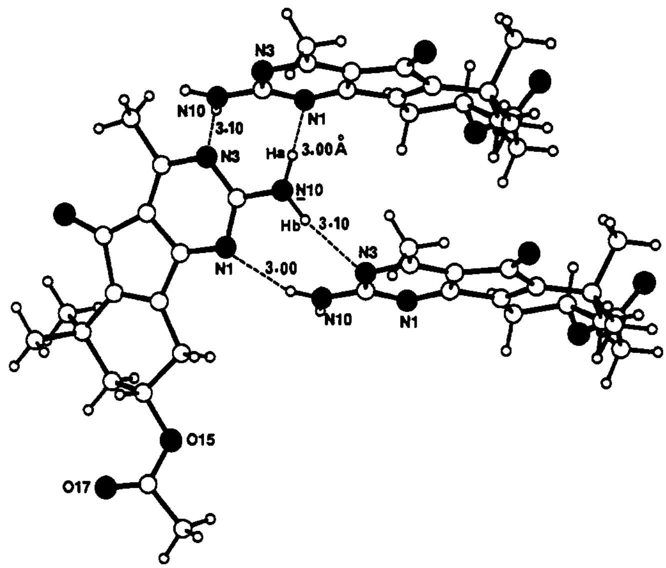


FIGURE 3. Drawing of three molecules in the crystal of acetylmillaurine [**2**]. Intermolecular hydrogen bonds are indicated by dotted lines.

X-RAY CRYSTAL ANALYSIS.[2]—Millaurine [**1**]: $C_{14}H_{17}N_3O_2$, 0.5 CH_2Cl_2; mol wt 259.31+42.47; crystals obtained by slow crystallization of millaurine from MeOH/CH_2Cl_2; monoclinic system, space group *C2*; *Z*=8; *a*=27.605 (15), *b*=7.388 (3), *c*=20.183 (12) Å; β=132.52 (2)°; V=3033 (2) $Å^3$; D_c=1.32 $g{\cdot}cm^{-3}$; F(000)=1272; λ (CuKα)=1.5418 Å; μ=2.2 mm^{-1}; 2525 measured intensities, 1945 observed.

Data were collected on a Phillips PW 1100 diffractometer with graphite-monochromated CuKα radiation. From the 2447 reflections measured by the θ–2θ scan technique up to θ=60°, only 1956 were considered as observed and kept in refinement calculations having $I \geq 3\sigma(I)$, σ(*I*) from counting statistics.

Acetylmillaurine [**2**]: $C_{16}H_{19}N_3O_3$; mol wt 301.35; crystals obtained by slow crystallization of acetylmillaurine from EtOH; orthorhombic system, space group $P2_12_12_1$, *Z*=4, *a*=7.019 (3), *b*=13.110 (6), *c*=16.849 (8) Å; V=1550.4 (6) $Å^3$; D_c=1.29 $g{\cdot}cm^{-3}$; F(000)=640; λ (CuKα)=1.5418 Å; μ=0.70 mm^{-1}; 1348 measured reflections, 832 observed.

For both compounds, intensity data were measured on a Nonius CAD 4 diffractometer using graphite-monochromated CuKα radiation and the θ–2θ scan technique up to θ=60°. Only intensities with $I>3\sigma(I)$ were considered as observed and kept in refinement calculations, σ(I) being derived from counting statistics. Cell parameters were derived from the refinement of 25 well centered reflections.

The structures were solved by direct methods with the program SHELXS86 (9) and refined by full matrix least-squares minimizing the function $\Sigma w(F_o-|F_c|)^2$ with the program SHELX76 (10). The hydrogen atoms, located in difference Fourier maps, were introduced in the refinement at theoretical positions (C-H, N-H=1.00 Å) and assigned an isotropic thermal factor equivalent to that of the bonded atom, plus 10%. In the structure of **1**, two molecules of CH_2Cl_2 were found on two different binary axes. Convergence was reached at R=0.056 and R_w=0.071 for **1**, R=0.056 and R_w=0.080 for **2**, with $R_w=\{\Sigma w(F_o-|F_c|)^2/\Sigma wF_o^{\,2}\}^{1/2}$ and $w=1/[\sigma^2(F_o)+kF_o^{\,2}]$ (*k*=0.00085 for **1**, 0.00312 for **2**). No residual was higher than 0.35 e $Å^{-3}$ for **1** (0.24 for **2**) in the final difference map.

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[2]Atomic coordinates for these structures have been deposited with the Cambridge Crystallographic Data Centre, and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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