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STRUCTURE AND TRANSFORMATIONS OF THE ALKALOID SANGUILUTINE

JIRÍ DOSTÁL*, JIRÍ SLAVÍK, MILAN POTÁCEK†, RADEK MAREK†, VLADIMÍR SKLENÁR†, EDMOND DE HOFFMANN‡, RAOUL ROZENBERG‡, BERNARD TINANT§ and JEAN-PAUL DECLERCQ§

Department of Biochemistry, Faculty of Medicine, Masaryk University, Komenského nám. 2, CZ-662 43 Brno, Czech Republic; † Department of Chemistry, Faculty of Science, Masaryk University, CZ-611 37 Brno, Czech Republic; ‡ Laboratoire de Spectrométrie de Masse, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium; § Laboratoire de Chimie Physique et de Cristallographie, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium

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Key Word Index—*Sanguinaria canadensis*; Papaveraceae; alkaloids; sanguilutine; free base; pseudobase; X-ray analysis.

Abstract—Sodium carbonate treatment of sanguilutine chloride yielded 6-hydroxy-5,6-dihydrosanguilutine. In a non-polar environment, 6-hydroxy-5,6-dihydrosanguilutine spontaneously transforms to *bis*[6-(5,6-dihydrosanguilutinyl)] ether, whose structure was confirmed by X-ray analysis. Treatment of sanguilutine chloride with aqueous ammonia gave *bis*[6-(5,6-dihydrosanguilutinyl)]amine. Reaction of sanguilutine chloride with potassium cyanide yielded 6-cyanodihydrosanguilutine; reaction of *bis*[6-(5,6-dihydrosanguilutinyl)]amine with ethanol gave 6-ethoxydihydrosanguilutine. © 1998 Published by Elsevier Science Ltd. All rights reserved

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INTRODUCTION

Sanguilutine (**1**), a pentamethoxy quaternary benzo[*c*]phenanthridine alkaloid, was isolated for the first time by Slavík, and Slavíková [1] from *Sanguinaria canadensis*. An attempt to determine the position of the fifth methoxyl group was made on the basis of mass and NMR spectroscopy of its cyano adduct [2]. The 2,3,7,8,10-substitution pattern has been unambiguously established by the total synthesis of sanguilutine [3, 4]. Hanaoka developed a biomimetic route-based synthesis of sanguilutine from a pentasubstituted protoberberine [5] and a tetrasubstituted benzophenanthridine [6]. Kim and Stermitz isolated dihydrosanguilutine from *S. canadensis* and provided its spectral data [7]. Recently [8], sanguilutine has been detected by HPLC in fresh methanol extracts of *Chelidonium majus*. Continuing our research on quaternary benzo[*c*]phenanthridine bases [9–11], we now present a detailed study on sanguilutine base.

RESULTS AND DISCUSSION

To quaternary golden-yellow sanguilutine chloride (**1**) dissolved in water, Na_2CO_3 was added to yield a white precipitate which was collected (method A). Its IR spectrum showed a strong and sharp absorption band of a semiaminal hydroxyl group at 3478 cm^{-1}. The ^1H NMR spectrum revealed that the product was 6-hydroxy-5,6-dihydrosanguilutine (**2a**), accompanied by a very small amount of *bis*[6-(5,6-dihydrosanguilutinyl)] ether (**3**); compound **2a** and similar species are called pseudobases [12–14]. In $CDCl_3$ solution, the composition of the product isolated gradually changed. After a few minutes, a mixture containing **2a** and **3** was observed. The signals of **2a** slowly decreased and, after 2 days, compound **3** was the major product recorded in the NMR spectrum. The structure of pseudobase **2a** was proven by several NMR experiments. The signal at δ 2.23 was found to be thermally labile and belongs to the semiaminal OH group. The signal is split by 4 Hz and the same coupling constant was found for the H-6 signal. Moreover, a clear interaction of OH and H-6 was observed in the COSY spectrum. Finally, the correlation of the OH hydrogen signal with C-6 and C-6a was determined in an HMBC experiment [15]. The chemical shifts of the H-6, 7-OMe and 8-OMe groups in **2a** are very close to those in chelerythrine pseudobase, which, on the contrary, arose due to hydrolysis of the O-bridged dimer base [9] (Table 1). The EI mass spectrum of **2a** showed an $[M]^+$ at *m/z* 411, of weak to moderate intensity, accompanied by fragment ions at *m/z* 394 $[M\text{-}OH]^+$, 379 and 364. The latter two fragments are formed either by successive loss of a

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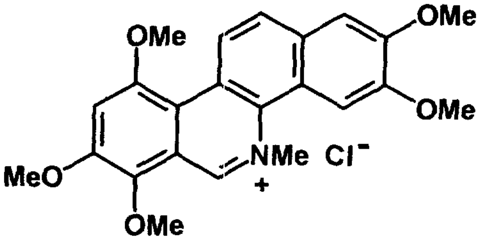
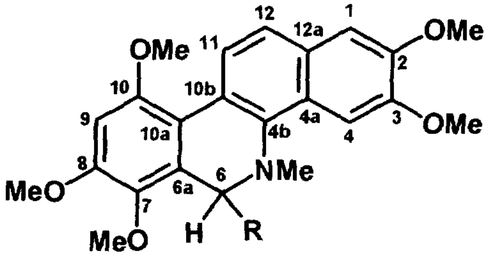
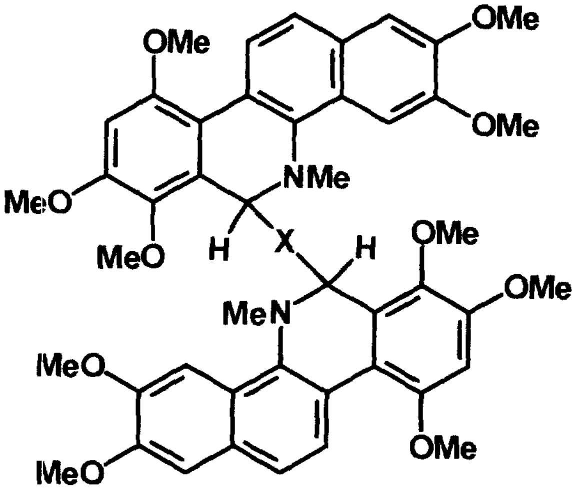
* Author to whom correspondence should be addressed.



1



2a: R = OH
2b: R = CN
2c: R = OEt



3: X = O
4: X = NH

methyl group or by loss of formaldehyde. MS/MS of the $[M]^+$ afforded the cation of the quaternary alkaloid **1** at *m/z* 394 as base peak. In the ESI spectrum, the molecular species of lower intensity was recorded with the *m/z* 394 ion as base peak. However, desorption chemical ionization performed with solid sample introduction proved the presence of a dimeric species **3** with low abundance. In the tandem mass spectrum of the $[M]^+$ at *m/z* 804, the *m/z* 394 ion was base peak, in addition to the *m/z* 410 (**1** + O) peak. Thus, DCI as a considerably softer technique confirmed the NMR observations.

The ^{1}H NMR spectrum of **3** displayed some typical features (Table 2). The rather low-field singlet of H-6 (6.54), the highly shielded singlet of 7-OMe (δ 2.28) and the slightly deshielded NMe signal (δ 3.06) are the apparent markers due to dimer structure effect. The addition of Na_2CO_3 to sanguilutine, followed by

Table 1. ^{1}H and ^{13}C NMR data of compounds **2a–c***

Position	**2a**		**2b**		**2c**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	7.13 *s*	106.6	7.13 *s*	106.6	7.13 *s*	106.8
2		149.4		149.8		149.2
3		149.4		149.8		149.2
4	7.63 *s*	102.7	7.63 *s*	102.4	7.67 *s*	102.8
4a		125.4		124.9		125.2
4b		137.6		138.3		138.3
6	6.00 *d* (4.2)	79.0	5.65 *s*	48.8	5.67 *s*	84.7
6a		128.0		123.0		128.3
7		140.4		139.8		140.5
8		153.7		153.6		153.8
9	6.67 *s*	98.3	6.67 *s*	98.6	6.64 *s*	99.6
10		152.2		152.5		152.2
10a		112.6		113.2		113.5
10b		121.2		121.9		121.8
11	8.46 *d* (8.8)	124.4	8.36 *d* (8.8)	124.2	8.47 *d* (8.8)	124.7
12	7.51 *d* (8.8)	122.2	7.56 *d* (8.8)	123.4	7.49 *d* (8.8)	121.8
12a		129.3		129.5		129.3
2-OMe	4.02 *s*	55.9	4.02 *s*	55.9	4.01 *s*	55.9
3-OMe	4.07 *s*	55.9	4.07 *s*	55.9	4.06 *s*	55.9
7-OMe	3.96 *s*	62.1	3.95 *s*	61.5	3.93 *s*	61.9
8-OMe	3.96 *s*	56.1	3.96 *s*	56.2	3.94 *s*	56.3
10-OMe	3.96 *s*	56 2	3.97 *s*	56.0	3.95 *s*	56.1
NMe	2.70 *s*	39.5	2.62 *s*	40.5	2.73 *s*	39.8
OH	2.23 *d* (4.2)					
CN				118.3		
O-CH$_2$					3.67–3.91 *m*	61.8
C-Me					1.05 *t* (7.0)	15.2

* Recorded at 500 MHz (^{1}H) and 125.7 MHz (^{13}C) in CDCl$_3$; coupling constants (Hz) in parentheses.

Et$_2$O extraction (method B), afforded a product whose IR, ^{1}H NMR, and mass spectral data were similar to those obtained by method A.

Compound **3** with two stereogenic carbon atoms can exist as a *meso*-form (6*R*,6′*S* ≡ 6*S*,6′*R*) and a racemate (6*S*,6′*S* + 6*R*,6′*R*). In contrast to our observations on sanguinarine [10] and chelirubine [16] free bases, only one diastereomer was detected. Recently, based on AM1 quantum chemistry calculations, Marek *et al.* [16] have suggested a racemate as a major stereoisomer for benzophenanthrine dimeric derivatives with a synclinal conformation in solution. The stereochemical arrangement is now supported by X-ray structural analysis and specified for the solid state. The method B sanguilutine free base was recrystallized from benzene and after 3 days afforded crystals suitable for X-ray data collection. The X-ray analysis revealed that the product obtained was *bis*(dihydrosanguilutinyl) ether (**3**); Fig. 1 shows a view of the molecule. Thus, spontaneous dimer formation in a non-polar environment is in agreement with the NMR observations. This is the first report of X-ray data of such an unusual bis-aminoacetal system. So far the evidence of dimeric constitution has been based on elemental analysis [9, 10], mass spectrometry [9, 10] and, recently, on pulsed-field-gradient HMBC [11] and GSQMBC [16, 17] NMR methods.

Compound **3** co-crystallized with two molecules of benzene. According to the centrosymmetric space group, the crystal is the racemic mixture of **3**. Selected geometrical parameters are presented in Table 3. The bond lengths C6-O18 (1.433 Å), O18-C6′ (1.438 Å), and the bond angle C6-O18-C6′ (113.2°), confirm the sp^3-hybridization of the oxygen connecting both parts of the dimeric structure. The conformation of the non-aromatic six-membered ring is a half-chair in both parts of the dimer, *viz*: ΔC2 (N5-C6) = 3.9 and 0.9° [18]. In each part of the dimer, four of the methoxyl groups are oriented in the plane of the ring; the fifth one (O15-C22) is nearly perpendicular (see torsion angles in Table 3). In the case of the in-plane conformation, the valence angles around the oxygen atoms are significantly enlarged; the mean value of C-O-CH$_3$ for the eight in-plane methoxyl substituents is 118.0°, compared with the average C-O15-C22 angles of 114.1°. This is probably a consequence of steric hindrance.

Treatment of quaternary sanguilutine (**1**) with aqueous ammonia, followed by diethyl ether extraction, yielded crystalline *bis*[6-(5,6-dihydrosanguilutinyl)]amine (**4**) as a single species. The IR spectra of **4**, even after repeated measurement, did not show an NH vibration band near 3300 cm^{-1}, as was observed in the case of chelerythrine [9] and sanguinarine [10].

Table 2. ^1H and ^{13}C NMR data of dimeric compounds **3** and **4***

	3		**4**		
Position	^1H	^{13}C	^1H	^1H†	^{13}C
1,1′	7.17 *s*	106.6	7.16 *s*	7.34 *s*	106.6
2,2′		149.5			149.4
3,3′		149.4			149.4
4,4′	7.98 *s*	103.0	8.05 *s*	7.95 *s*	103.2
4a,4a′		125.2			126.1
4b,4b′		137.8			138.9
6,6′	6.54 *s*	77.7	5.91 *s*	5.77 *s*	64.4
6a,6a′		128.0			130.5
7,7′		140.3			139.9
8,8′		153.4			153.3
9,9′	6.46 *s*	97.9	6.41 *s*	6.62 *s*	97.2
10,10′		152.1			152.0
10a,10a′		113.9			113.6
10b,10b′		122.1			122.2
11,11′	8.39 *d* (8.7)	124.5	8.33 *d* (8.8)	8.24 *d* (8.8)	124.5
12,12′	7.46 *d* (8.7)	121.7	7.45 *d* (8.8)	7.48 *d* (8.8)	121.6
12a,12a′		129.6			129.6
2-OMe,2′-OMe	4.05 *s*	55.8	4.05 *s*	3.95 *s*	55.8
3-OMe,3′-OMe	4.12 *s*	55.8	4.13 *s*	4.00 *s*	55.8
7-OMe,7′-OMe	2.28 *s*	60.6	2.43 *s*	2.31 *s*	60.5
8-OMe,8′-OMe	3.85 *s*	56.3	3.84 *s*	3.84 *s*	56.2
10-OMe,10′-OMe	3.74 *s*	55.6	3.74 *s*	3.70 *s*	55.6
NMe,N′Me	3.06 *s*	39.9	2.92 *s*	2.86 *s*	40.3
NH				1.15 *br s*	

* For measurement conditions see Table 1.
† In DMSO-d_6.

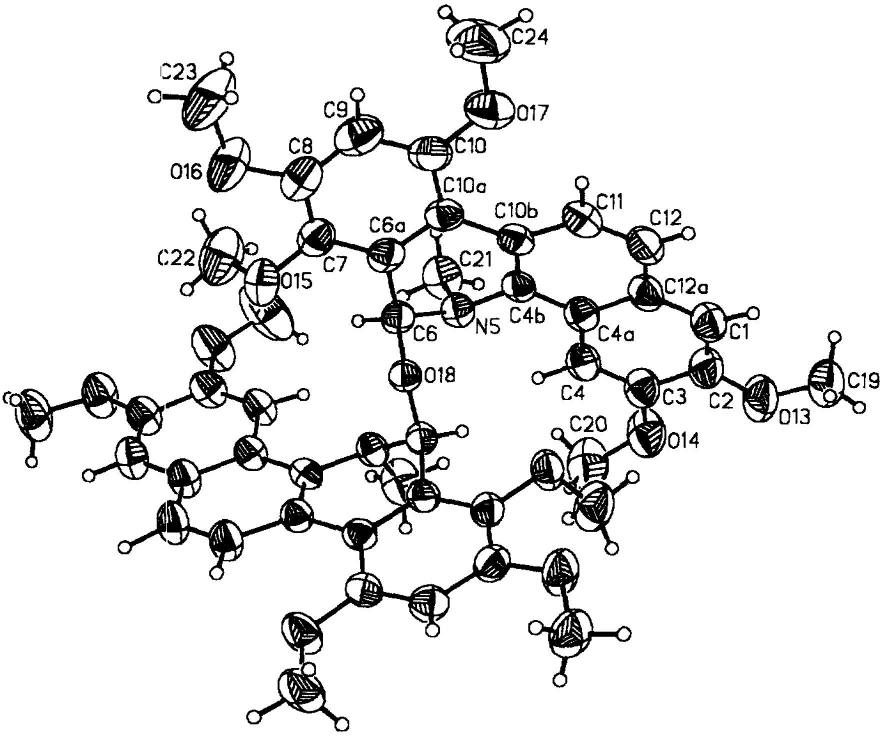


Fig. 1. View of molecule **3** with partial atom numbering.

Table 3. Selected geometrical parameters of compound **3***

Bond lengths (Å)		
C(1)—C(2)	1.362(5)	1.357(5)
C(1)—C(12A)	1.427(5)	1.420(4)
C(2)—O(13)	1.366(4)	1.357(4)
C(2)—C(3)	1.435(5)	1.424(5)
C(3)—O(14)	1.362(4)	1.375(4)
C(3)—C(4)	1.367(5)	1.364(4)
C(4)—C(4A)	1.421(5)	1.430(4)
C(4A)—C(12A)	1.419(4)	1.418(4)
C(4A)—C(4B)	1.421(4)	1.421(4)
C(4B)—C(10B)	1.386(4)	1.388(4)
C(4B)—N(5)	1.430(4)	1.423(3)
N(5)—C(6)	1.443(4)	1.438(4)
N(5)—C(21)	1.459(4)	1.454(4)
C(6)—O(18)	1.433(3)	1.438(5)
C(6)—C(6A)	1.506(4)	1.504(4)
C(6A)—C(7)	1.389(4)	1.373(4)
C(6A)—C(10A)	1.400(4)	1.405(4)
C(7)—C(8)	1.369(5)	1.400(4)
C(7)—O(15)	1.399(4)	1.391(3)
C(8)—O(16)	1.377(4)	1.363(4)
C(8)—C(9)	1.379(6)	1.371(4)
C(9)—C(10)	1.395(5)	1.385(4)
C(10)—O(17)	1.382(4)	1.373(3)
C(10)—C(10A)	1.402(5)	1.408(4)
C(10A)—C(10B)	1.488(4)	1.487(4)
C(10B)—C(11)	1.422(4)	1.431(4)
C(11)—C(12)	1.365(5)	1.365(5)
C(12)—C(12A)	1.400(5)	1.411(4)
O(13)—C(19)	1.408(5)	1.413(5)
O(14)—C(20)	1.417(5)	1.358(5)
O(15)—C(22)	1.423(5)	1.415(5)
O(16)—C(23)	1.409(5)	1.429(4)
O(17)—C(24)	1.423(5)	1.422(4)
Bond angles (°)		
C(4B)—N(5)—C(6)	114.2(2)	115.3(2)
C(4B)—N(5)—C(21)	115.1(2)	115.3(2)
C(6)—N(5)—C(21)	113.4(2)	114.0(2)
C(2)—O(13)—C(19)	116.9(3)	117.4(3)
C(3)—O(14)—C(20)	116.9(3)	119.1(3)
C(7)—O(15)—C(22)	115.4(3)	112.8(3)
C(8)—O(16)—C(23)	118.5(4)	117.8(3)
C(10)—O(17)—C(24)	118.7(4)	118.8(3)
C(6)—O(18)—C(6′)	113.9(2)	
Torsion angles (°)		
N(5)—C(6)—O(18)—C(6′)	−53.3(3)	−53.7(3)
C(6A)—C(6)—O(18)—C(6′)	−176.0(2)	−177.0(2)
C(1)—C(2)—O(13)—C(19)	0.8(6)	4.1(6)
C(4)—C(3)—O(14)—C(20)	−14.1(6)	−13.6(6)
C(8)—C(7)—O(15)—C(22)	75.9(4)	79.6(4)
C(9)—C(8)—O(16)—C(23)	2.5(6)	4.9(5)
C(9)—C(10)—O(17)—C(24)	1.4(6)	5.0(5)

* Data in right column refer to unmarked half of structure.

In DMSO-d_6, however, the NH group of **4** appeared as a signal at δ 1.15, detected by interaction with H-6 in DQF COSY using pulsed-field gradients [19]. Its integral intensity of 0.5 conforms with the dimer structural concept. The positive DCI mass spectrum of **4** showed the molecular cation-radical at *m/z* 803 and a peak at *m/z* 409 corresponding with a **1**+NH fragment. In the negative DCI mode, the $[M\text{-}H]^-$ ion at *m/z* 802 appeared with significantly greater intensity. The tandem mass spectrum of the *m/z* 802 ion displayed intense peaks at *m/z* 394, 380 and 364, in agreement with the expected fragmentation of **1**.

HMBC experiments have established the dimeric nature of compounds **3** and **4**. An HMBC experiment optimized for long-range coupling of 7.5 Hz using gradient pulses (gradient ratio 50, 30 and 40) for coherence selection and without low-pass J-filter was carried out. The resulting spectrum displayed one bond coupling of H-6 with C-6 (160 and 153 Hz for **3** and **4**, respectively), as well as long-range interaction [11] of H-6 with C-6′. In the ^{1}H, ^{15}N-HMBC spectrum [20], a correlation between the H-6 and hydrogen atoms in CH_3N with the nitrogen atom at δ 34.21 appeared.

The 6-cyano adduct **2b** was prepared almost quantitatively by direct nucleophilic addition [13] of CN^- to the quaternary cation **1**. In the IR spectrum, the cyano group showed a very weak absorption at 2232 cm^{-1}. The N atom attached at the α-position to the CN group reduces considerably the intensity of IR absorption. 6-Ethoxydihydrosanguilutine (**2c**) was obtained by recrystallization of **3** or **4** from EtOH. By treatment with dilute acids, compounds **2a**, **2c**, **3** and **4** immediately gave bright yellow quaternary sanguilutine salts of the **1**-type.

In order to obtain complete ^{1}H and ^{13}C NMR assignments of compounds **2**–**4**, a series of 1D and 2D NMR experiments was undertaken. ^{1}H NMR signals were assigned in NOESY experiments [21]. The chemical shifts of all protonated carbon atoms were determined by HSQC pulse sequence [22]. For assignment of quaternary carbon atoms, HMBC [15] and GSQMBC [17] pulse-sequences optimized for long-range coupling constants of 11, 7 and 4 Hz were used. In the HSQC, HMBC and GSQMBC, gradient-pulses [23] were used for coherence selection. The hydrogen atom signals of the OMe groups were assigned by NOESY experiments showing interactions with the skeleton protons. HMBC clearly showed interactions of the OMe hydrogen atoms with the C-2, C-3, C-7, C-8 and C-10 quaternary carbon atoms. Moreover, when optimizing the long-range coupling to 4 Hz, interactions with C-1, C-4 and C-9 were observed.

EXPERIMENTAL

Mps: uncorr. IR: KBr discs. NMR: 500 MHz for ^{1}H, 125 MHz for ^{13}C, in $CDCl_3$, unless otherwise stated; TMS int. standard. EIMS: 70 eV. CIMS: CH_4-N_2O ionizing gas. ESIMS: MeCN. Sanguilutine chloride (**1**) (golden-yellow needles, mp 159–160°) was isolated from rhizomes of *Sanguinaria canadensis* L. as described previously [1]. The Et_2O used was EtOH-free.

6-*Hydroxy*-5,6-*dihydrosanguilutine* (**2a**). *Method A*. Sanguilutine chloride (**1**) (71 mg) was dissolved in H_2O

and the soln made alkaline with satd aq. Na_2CO_3. The white ppt. formed was allowed to stand for 15 min at room temp., then filtered off, washed with H_2O and dried *in vacuo* (53 mg). Mp 240–243°. IR ν^{KBr} 3478 (s, $\Delta\nu_{1/2} = 37$ cm^{-1}, OH), 3089, 2990, 2930, 2839, 1626, 1600, 1562, 1500, 1466, 1434, 1377, 1330, 1251, 1163, 1134, 1084, 1060, 1030, 983, 945, 862 cm^{-1}. ^{1}H and ^{13}C NMR ($CDCl_3$): Table 1. EIMS: *m/z* $[M]^{\cdot+}$ 411 (11), 395 (100), 394 (40), 379 (67), 364 (34), 336 (11), 320 (6); MS/MS (411): 394 (100), 364 (21). ESI (positive mode): *m/z* $[M]^{\cdot+}$ 411 (2), $[M+H-H_2O]^+$ 394 (100), 379 (5), 364 (7). MS/MS: *m/z* (411): 410 (9), 395 (93), 394 (100), 380 (83), 379 (26), 364 (10). DCI (positive mode): *m/z* $[M]^{\cdot+}$ of **3** 804 (9), 802 (6), 789 (10), 426 (3), $[M+H]^+$ of **2a** 412 (27), 394 (100), 380 (35); MS/MS *m/z* (804): 410 (1), 394 (100); MS/MS *m/z* (412): 412 (100), 397 (60), 394 (34), 380 (9), 364 (4). *Method B*. Sanguilutine chloride (**1**) (80 mg) was dissolved in H_2O and the soln made alkaline with satd aq. Na_2CO_3. The ppt. was extracted with Et_2O and the organic layer concd and allowed to stand at room temp. After 5 hr, colourless crystals were collected and dried *in vacuo* (43 mg). Mp 244–246°. IR (KBr) and ^{1}H NMR ($CDCl_3$) data were identical to those of the method A product. DCI (positive mode) *m/z* $[M]^{\cdot+}$ of **3** 804(5), 789(2), 422(2), $[M+H]^+$ of **2a** 412(17), 411(9), 394(100).

X-Ray analysis. Method B free base was recrystallized from C_6H_6 to give crystals of **3**. Crystallographic data are as follows: $C_{46}H_{48}N_2O_{11}\cdot 2C_6H_6$, $M_r = 961.12$, triclinic, P-1, $a = 12.898(1)$, $b = 14.264(2)$, $c = 16.062(2)$ Å, $\alpha = 102.35(1)°$, $\beta = 99.21(1)°$, $\gamma = 111.69(1)°$, $V = 2587.5(5)$ Å^3, $Z = 2$, $D_x = 1.23$ g cm^{-3}. Parallelepiped crystal with dimensions $0.10 \times 0.20 \times 0.32$ mm. Lattice parameters were refined using 30 reflections in the range $15° \leqslant 2\theta \leqslant 50°$. Huber four-circle diffractometer and Rigaku rotating anode, graphite monochromatized CuKα radiation ($\lambda = 1.5418$ Å). 9327 measured reflections, with $\sin\theta/\lambda \leqslant 0.60$ Å^{-1}; $0 \leqslant h \leqslant 15$, $-17 \leqslant k \leqslant 15$, $-19 \leqslant l \leqslant 18$, 5892 with $I \geqslant 2.0\sigma(I)$. A standard reflection ($3\ -1\ -2$) was checked every 50 reflections; no significant deviation was observed. The structure was solved by direct methods using SHELXS86 [24]. All ring H atoms were located from difference Fourier synthesis; the other ones (methyl groups, H of C_6H_6) were calculated with AFIX. Anisotropic least squares refinement (SHELXL93) [25] using F; H isotropic with common refined temperature factor ($U = 0.13$ Å^2). 653 parameters. Restraints (regular hexagon with C-C distances of 1.39 Å) were applied to the two solvent molecules of C_6H_6 $w = 1/(\sigma^2(F_o)^2 + 0.1585P^2)$, $R = 0.077$, 0.107 (all data), $S = 1.11$. Final maximum shift to error = 0.1. Maximum and minimum heights in final difference Fourier synthesis = 0.55 and -0.38 e Å^{-3}. Complete X-ray data have been deposited at the Cambridge Crystallographic Data Centre.

6-*Cyano*-5,6-*dihydrosanguilutine* (**2b**). Sanguilutine chloride (**1**) (60 mg) was dissolved in H_2O and an excess of aq. NaCN was added to the soln. A white ppt. was sepd, dried and recrystallized from $CHCl_3$–EtOH (1:10); yield 56 mg (97%). Mp 231–232°. IR ν^{KBr} cm^{-1}: 3087, 3005, 2943, 2838, 2232 (w, CN), 1626, 1598, 1562, 1500, 1464, 1429, 1371, 1331, 1261, 1205, 1156, 1083, 1026, 1005, 976, 861 and 826. ^{1}H and ^{13}C NMR ($CDCl_3$): Table 1. DCI MS (positive mode): *m/z* $[M+H]^+$ 421 (21), $[M]^{\cdot+}$ 420 (21), 394 (100), 378 (4), 279 (2); MS/MS *m/z* (421): 406 (17), 394 (100), 364 (44). DCI (negative mode): *m/z* $[M\text{-}H]^-$ 419 (100), 394 (40), 364 (60). Found: C, 68.45; H, 5.89; N, 6.61. Calc for: $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66%.

6-*Ethoxy*-5,6-*dihydrosanguilutine* (**2c**). The compound was obtained after repeated recrystallization of **4** from $CHCl_3$–EtOH (1:10). Mp 237–238°. IR ν^{KBr} cm^{-1}: 3087, 2969, 2936, 2838, 1626, 1600, 1562, 1499, 1463, 1436, 1406, 1333, 1251, 1220, 1202, 1163, 1066, 1027, 1006, 986, 965, 863 and 830. ^{1}H and ^{13}C NMR ($CDCl_3$): Table 1. DCI MS (positive mode): *m/z* $[M+H]^+$ 440 (3), $[M]^{\cdot+}$ 439 (3), 424 (9), 410 (9), 396 (82), 395 (100), 380 (47); MS/MS (440) 394 (100), 380 (88), 364 (56). DCI (negative mode): *m/z* $[M\text{-}H]^-$ 438 (5), 422 (5), 408 (20), 394 (100), 378 (30), 364 (25). Found: C, 68.42; H, 6.41; N, 3.20. $C_{25}H_{29}NO_6$ requires: C, 68.32; H, 6.65; N, 3.19%.

Bis[6-(5,6-*dihydrosanguilutinyl*)]*amine* (**4**). Sanguilutine chloride (**1**) (102 mg) was dissolved in H_2O and the soln made alkaline with conc aq. NH_3. The ppt. was extracted with Et_2O, the organic layer concentd and allowed to stand at room temp. After 3 hr, colourless crystals were collected and dried *in vacuo* (78 mg, 82%). Mp 249–251°. IR ν^{KBr} cm^{-1}: 3084, 2996, 2937, 2835, 1625, 1597, 1562, 1495, 1463, 1431, 1404, 1328, 1263, 1220, 1202, 1160, 1084, 1026, 1001, 981, 862 and 835. ^{1}H and ^{13}C NMR ($CDCl_3$), ^{1}H NMR (DMSO-d_6): Table 2. DCI MS (positive mode): *m/z* $[M+H]^+$ 804 (10), $[M]^{\cdot+}$ 803 (14), 789 (1), 410 (3), 394 (100), 380 (4); MS/MS (803): 409 (45), 394 (100), 364 (5). DCI (negative mode): *m/z* $[M\text{-}H]^-$ 802 (25), 409 (31), 394 (100), 378 (49), 364 (18); MS/MS *m/z* (802): 394 (100), 380 (85), 364 (85). Found: C, 68.22; H, 5.96; N, 5.31. $C_{46}H_{49}N_3O_{10}$ requires: C, 68.73; H, 6.14; N, 5.23%.

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