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THE STRUCTURE OF ISOSTRYCHNOPENTAMINE, A BISINDOLE MONOTERPENE ALKALOID FROM *STRYCHNOS USAMBARENSIS*

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Key Word Index—*Strychnos usambarensis*; Loganiaceae; alkaloids; chemical constitution; configuration; isostrychnopentamine.

Abstract—The alkaloid isostrychnopentamine has been shown to be epimeric with strychnopentamine at the asymmetric carbon atom of the *N*-methylpyrrolidin-2-yl group. Its IUPAC name is 2-[(1′,2′,3′,4′-tetrahydro-2′-methyl-β-carbolin-1′-yl)methyl]-11-(1″-methyl-pyrrolidin-2″-yl)-3-vinyl-1,2,3,4,6,7,12,12b-octahydro-indolo[2,3-a]quinolizin-10-ol [2(*S*),3(*R*),12b(*S*),1′(*S*),2″(*S*)].

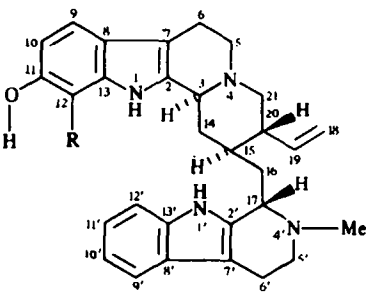
INTRODUCTION

The leaf of the African plant *Strychnos usambarensis* contains numerous bisindole monoterpene alkaloids among which is the peculiar strychnopentamine [1]. This compound presents considerable pharmacological interest because of its potent antimitotic properties [2]. The structure of strychnopentamine (**1**) was firmly established by crystallographic methods in 1977 [3]. Its distinctive feature is the unprecedented joining of a pyrrolidine ring to the widely distributed β-carbolinyl indolo[2,3-a]-quinolizinyl-methane system (resulting in a molecule with five nitrogen atoms, hence the name strychnopentamine). Two more compounds from *S. usambarensis*, probably related to strychnopentamine, were mentioned briefly by the Liège authors in 1977 and 1978 [1, 3]. We now report that isostrychnopentamine (**2**), designated in 1978 as isostrychnopentamine A (Fig. 1), is the C-2″ epimer of strychnopentamine.

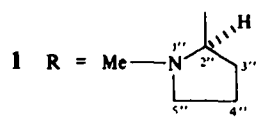
RESULTS AND DISCUSSION

The IR, UV, 90 MHz ^{1}H NMR and mass spectra of strychnopentamine and isostrychnopentamine ($C_{35}H_{43}N_5O$, M_r 549) are almost indistinguishable, as are the CD curves. The ^{13}C NMR spectra of the two alkaloids are practically superimposable. However, the 360 MHz ^{1}H NMR spectra showed for some hydrogens minor differences in chemical shift, which it was felt could provide a basis for structural discrimination. We therefore undertook the arduous task of assigning the complex ^{1}H NMR spectra, relying for that purpose on an extensive series of 1D- and 2D-experiments. The ^{1}H NMR parameters obtained are listed in Table 1. We stress that the assignments are based solely on information provided by the spectra and by the X-ray data of strychnopentamine, except for the two NMe groups. Here, the one of higher frequency was assigned to the NMe group of the β-carboline system, by analogy with known compounds [4].

The hydrogens bonded to C-21–C-20 (C-19=C-18)–C-15 (C-16–C-17)–C-14–C-3 form a ^3J contiguous set in the sense that there is at least one non-zero ^3J H–H coupling constant along each C–C bond. Two methylenes of this set, (14) CH_2 and (16) CH_2, are not α to nitrogen or α to a double bond, and consequently absorb in the uncluttered lower frequency part of the spectrum. They are easily discriminated by the magnitude of their ^2J coupling constant, *ca* −12 Hz for (14) CH_2 (C-15–C-14–C-3 valency angle equals 111°) and *ca* −14.4 Hz for (16) CH_2 (the C-15–C-16–C-17 valency angle is large, 119°, hence a negative contribution to ^2J). Starting off from these now



1 R = Me—N



2 R = Me—N

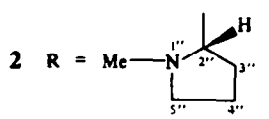


Fig. 1. Structures and phytochemical numbering of strychnopentamine (**1**) and isostrychnopentamine (**2**).

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Table 1. Chemical shifts of aliphatic hydrogens* in compounds 1 and 2 (360 MHz, $CDCl_3$, TMS as internal standard)

H	1	2	H	1	2
3	3.12	3.13	6'A	2.84	2.85
6A	2.83	2.85	6'B	2.84	2.85
6B	2.59	2.59	5'A	3.20	3.20
5A	3.00	3.00	5'B	2.74	2.76
5B	2.49	2.49	N-4' Me	2.47	2.45
14e	1.76	1.82			
14a	1.14	1.14	2"	3.19	3.20
15	1.87	1.88	3"A	1.97	2.13
16A	2.27	2.28	3"B	1.75	1.77
16B	1.58	1.57	4"A	1.97	1.94
17	3.60	3.65	4"B	1.97	1.77
20	2.27	2.28	5"A	3.31	3.31
21e	2.92	2.93	5"B	2.40	2.52
21a	2.27	2.28	N-1" Me	2.27	2.33

*Aromatic hydrogens (1,2) H-10 = δ6.60; H-9 = δ7.13, $J_{H\text{-}10,H\text{-}9}$ = 8.4 Hz; H-12', H-9', H-10', H-11' at 7.50 (*d*), 7.31 (*d*), 7.13 (*m*) and 7.11 (*m*).

a, Axial; e, equatorial; A and B, low-field and high-field resonances, respectively, of a methylene group.

assigned methylenes, one finds directly three vicinal methine protons: H-17 (at δ3.60 it has the highest frequency of the hydrogens bonded to saturated carbon), H-3 (δ3.12) and H-15 (δ1.87), double irradiation of which affects the four methylene hydrogens. In a similar way, one finds from the constitutionally unique vinylic hydrogen H-19, which lies all alone at a characteristic position in the spectrum, the methine H-20 and from there the methylene hydrogens (21) CH_2.

The $-CH_2CH_2-$ groups of the indoloquinolizine and the β-carboline moiety can be distinguished due to a homoallylic ^5J coupling between H-3 and (6)CH_2, respectively, and between H-17 and (6')CH_2. Characteristically, the diastereotopic (5)CH_2 hydrogens, situated near the 'asymmetric nitrogen' N-4 have a sizeable chemical shift difference (*ca* 0.5 ppm) and enclose between them the (6)CH_2 hydrogens.

Changes in configuration of the stereogenic atoms C-3, C-15, C-20, C-17 and N-4 of various indoloquinolizine alkaloids and usambarine derivatives result in significant changes in the coupling constant and chemical shift [4]. Inspection of Table 1 shows that the indoloquinolizine and β-carboline regions of strychnopentamine and isostrychnopentamine have practically the same ^{1}H NMR parameters. For instance, the chemical shift difference for 17 out of the 19 corresponding hydrogens is equal to or less than 0.02 ppm, and the largest difference (H-14 eq) is a mere 0.06 ppm. Thus, strychnopentamine and isostrychnopentamine are identical in the β-carboline and indoloquinolizine region and must be different in the pyrrolidine moiety.

The hydrogen atoms of the pyrrolidine ring were difficult to assign. In strychnopentamine, only two out of the seven hydrogens lie isolated in the spectrum: (i) a broadened triplet, splitting *ca* 8 and 9 Hz, at δ3.31 and (ii) a broadened quartet, splitting *ca* 9 Hz, at δ2.40, just between the two NMe resonances. A 2D COSY 45° experiment [5] established, by the ellipticity of the two cross-peaks emanating from the triplet at δ3.31, that the 8 and 9 Hz coupling constants have opposite signs. This means that one is a geminal coupling and the other a vicinal coupling. The triplet at δ3.31 is therefore due to one of the (5")CH_2 methylene hydrogens, with one of its two vicinal couplings small. Incidentally, the other (5")CH_2 hydrogen is the quartet-like signal at δ2.40. The signal of hydrogen H-2" lies at δ3.19, overlapping with H-5'A. The (3")CH_2 and (4")CH_2 methylene hydrogens all lie in the low frequency part of the spectrum, H-3"B at δ1.75, and the other three at δ1.97.

In isostrychnopentamine, three out of the seven pyrrolidinyl resonances are reasonably isolated: H-5"A at δ3.31, H-5"B at δ2.52 and H-3"A at δ2.13. On comparing the chemical shift of the corresponding pyrrolidinyl hydrogens of strychnopentamine and isostrychnopentamine, three differences in the range 0.1–0.2 ppm are found. Interestingly, the chemical shift of H-2", directly bonded to the chiral centre, is the same in both isomers, whereas differences are found for some of the remote hydrogens, including those of NMe.

Apart from the above-mentioned shift differences, the overall ^{1}H NMR characteristics of the 2-(*N*-methylpyrrolidinyl) group in strychnopentamine, isostrychnopentamine and nicotine [6] are strikingly similar: (1) large anisochronism of the (5")CH_2 hydrogens, with H-5"A (the one of higher frequency) having a large and a small vicinal coupling constant; (ii) H-2" somewhat more shielded than H-5"A, and (iii) small anisochronism of the (3")CH_2 and (4")CH_2 hydrogens. All of this suggests that in these compounds the 2-(*N*-methylpyrrolidinyl) group assumes the same conformation and the same orientation with respect to the aromatic ring.

From the tenets of conformational analysis one expects a roughly perpendicular orientation of the mean plane of the pyrrolidine and of the aromatic ring. The crystallographic data for strychnopentamine add more detail [3]. First, the C-2"–C-3" bond stands almost perpendicularly to and on the β-side of the indoloquinolizine plane, while the C-2"–N-1" and C-2"–H bonds stand very obliquely on the α-side (the half-space of indoloquinolizine that contains H-3 is by convention the α half-space). Secondly, N-1" and the hydroxyl on C-11 are engaged in intramolecular hydrogen bonding (the O ... N distance is 264 pm, and the nitrogen lone pair is properly orientated towards the hydroxyl group).

We have envisaged two possibilities for the relationship between strychnopentamine and isostrychnopentamine: (i) diastereomerism due to the opposite configuration of C-2", and (ii) diastereomerism due to hindered rotation of the C-12–C-2" bond. If it were hindered rotation, then the C-2"–H bond, which in strychnopentamine is extended towards N-1" would in isostrychnopentamine be extended towards HO (C-11). The identity in shift and in shape of the H-2" signal in both isomers contradicts and eliminates the hindered rotation hypothesis. In this latter case, we would notice a bathochromic shift in the UV spectrum of isostrychopentamine on passing from neutral to alkaline solution. However, no shift was seen in the spectrum of either alkaloid, both of which must therefore possess a cryptophenolic function. We must conclude that strychnopentamine and isostrychnopentamine differ in the configuration of C-2".

The following facts and reasoning support the hypothesis of the opposite configuration of C-2" in the two alkaloids. We shall assume that the solution conformation

of strychnopentamine, in particular the torsion angles around the C-15–C-16 and C-16–C-17 bonds, is the same as that in the crystal, or at least that the crystal conformation is significantly present in solution. The available vicinal coupling constants of the (16)CH_2 hydrogens are consistent with this assumption, but do not prove it. The crystal conformation of strychnopentamine has the shape of a slightly widened 'U' [3]. One leg of the 'U' contains the indoloquinolizine unit, the other leg the β-carboline unit, and in the bend lie the + 75°, − 63° torsion angles of the bond sequence C-14–C-15–C-16–C-17–C-2'. Within this 'U', the mean plane of the indoloquinolizine and of the β-carboline system makes an obtuse angle [3], such that the latter lies in the α half-space of the former. As a consequence, the aromatic system of the β-carboline is, if not close, at least in the neighbourhood of the 2-(*N*-methylpyrrolidinyl) group (the midpoints of the benzene ring from the β-carboline unit and of the pyrrolidine ring are *ca* 500 pm apart). A schematic representation is shown in Fig. 2; more elaborate drawings and a stereopsis set can be found in ref. [3]. The crux of the matter is that the N-1″ methyl group of strychnopentamine lies in the shielding zone of the aromatic ring current from the β-carboline moiety, whereas this is not or much less the case for the N-1″ methyl group of isostrychnopentamine.

Experimentally, the N-1″ methyl group is shielded by 0.06 ppm in strychnopentamine relative to isostrychnopentamine. A rough approximation using internal coordinates measured from a crude ball-and-stick model and Johnson's anisotropy tables for benzene gives a 0.1 ppm effect. The hydrogens of the N-1″ methyl group are sensitive to the above-discussed magnetic anisotropy (ring currents) effects because their spatial coordinates change so much on inverting the configuration of C-2″: they move over about 550 pm.

The ^{13}C chemical shifts of strychnopentamine and isostrychnopentamine are collected in Table 2. The identification of the non-protonated carbon atoms and of the NMe groups is based on data for analogous compounds. A series of single frequency off-resonance ^1H decoupled spectra has led to the assignments for the hydrogen-bearing carbon atoms shown in Table 2. The assignments so obtained were, in the case of strychnopentamine, fully confirmed by the ^{13}C–^1H shift correlation at 360 MHz. This correlation experiment confirms that the triplet-like ^1H resonance at δ3.31 belongs to (5″)CH_2, in agreement with our earlier discussion concerning the pyrrolidinyl hydrogen atoms. Thus, the near identity of the assigned ^{13}C NMR spectra of the two alkaloids **1** and **2** eliminates the last qualms about their possibly being epimeric at C-17 [4, 7, 8] and corroborates the conclusions drawn from the ^1H NMR parameters.

Table 2. ^{13}C NMR spectral data for compounds **1** and **2** (50.29 MHz, $CDCl_3$, TMS as internal standard)

sp^3 C	1	2	sp^2 C	1	2
3	59.79 *d*	59.70 *d*	2*	132.5 *s*	132.05 *s*
5	52.99 *t*	52.86 *t*	7†	106.4 *s*	106.2 *s*
6	21.66 *t*	21.43 *t*	8	120.8 *s*	120.4 *s*
14	35.78 *t*	35.25 *t*	9	117.28 *d*	117.2 *d*
15	36.20 *d*	35.81 *d*	10	110.64 *d*	110.6 *d*
16	36.35 *t*	35.66 *t*	11	152.8 *s*	152.71 *s*
17	59.02 *d*	58.9 *d*	12†	108.6 *s*	108.16 *s*
20	48.27 *d*	47.83 *d*	13*	135.7 *s*	135.2 *s*
21	61.48 *t*	61.03 *t*	18	116.80 *t*	117.2 *t*
N-4′ Me	42.41 *q*	42.03 *q*	19	140.51 *d*	140.1 *d*
5′	51.23 *t*	51.90 *t*	2′*	135.5 *s*	135.2 *s*
6′	20.03 *t*	19.86 *t*	7′†	108.5 *s*	107.8 *s*
N-1″ Me	40.16 *q*	40.02 *q*	8′	127.5 *s*	127.1 *s*
2″	65.77 *d*	65.38 *d*	9′	117.87 *d*	117.75 *d*
3″	31.82 *t*	32.05 *t*	10′	121.69 *d*	121.8 *d*
4″	23.08 *t*	22.96 *t*	11′	119.58 *d*	119.7 *d*
5″	55.91 *t*	55.40 *t*	12′	110.73 *d*	110.8 *d*
			13′*	136.0 *s*	135.9 *s*

*,†Assignments not firmly attributed; these values may be interchanged.

EXPERIMENTAL

Isostrychnopentamine was extracted from *S. usambarensis* Gilg leaves as described before [1] and obtained in a pure state by chromatography on silica gel, eluant EtOAc–*iso*-PrOH–NH_3 (45:4:1). It is eluted somewhat after strychnopentamine. The free base is a yellowish powder, soluble in $CHCl_3$ and MeOH, but also in boiling hexane from which it is precipitated on cooling. NMR spectra were run in $CDCl_3$, after removing paramagnetic impurities by treatment with Chelex^R(Bio-Rad Co.). Solns must be kept in a cool place and away from light because they turn brown on standing and deteriorate in sunlight.

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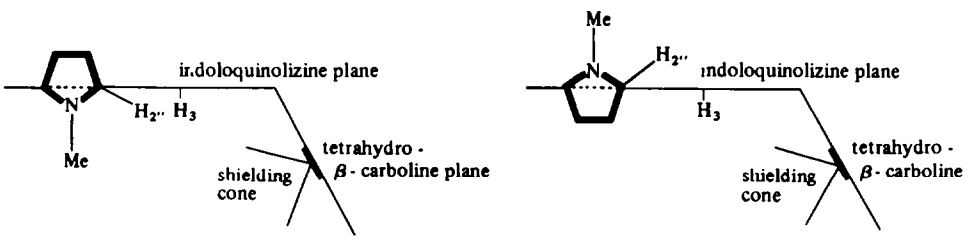

Fig. 2. Schematic representation of the position of the pyrrolidine ring and of the tetrahydro-β-carboline aromatic current in strychnopentamine (left) and isostrychnopentamine (right).

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