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TETRAHEDRON

New Indolines Derivatives from Strychnobrasiline, Modulators of Chloroquine Resistance in *Plasmodium falciparum*

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Abstract : an unexpected molecular rearrangement in lithium aluminium hydride reduction of the indole alkaloid strychnobrasiline was observed. The resulting derivatives obtained were evaluated as modulators of chloroquine resistance in *Plasmodium falciparum.*

INTRODUCTION

Infusion of *Strychnos myrtoïdes* Gilg & Buss (Loganiaceae) has been empirically used as chloroquine-adjuvant in malagasy herbal remedies for the treatment of chronic malaria. Strychnobrasiline,**1**, and malagashanine,**2**, (revised structure)[1], are the two major alkaloid constituents of *S. myrtoïdes.* It has been shown that **1** and **2** lacked both intrinsic antimalarial activity and cytotoxycity effect, but exhibited *in vitro* significant chloroquine potentiating action against a chloroquine resistant strain of *Plasmodium falciparum.*[2] Unfortunately *in vivo* activity of strychnobrasiline, which is the major alkaloid of the plant, has been found low compared to malagashanine.[2] It was therefore of interest to prepare malagashanine from strychnobrasiline.

While strychnobrasiline belongs to the known series Nb-C(3)-secocuran series of *Strychnos* alkaloids, malagashanine was the first Nb-C(21)-secocuran alkaloid so far described. A comparative structural examination of the two series suggests that such a conversion might take place via hydrolysis of the immonium salt. During our preliminary studies on the chemical reactivity of strychnobrasiline we observed that the treatment of **1** by $LiAlH_4$ gave rise to several new derivatives through unexpected rearrangement. In this paper we present the structural characterization and the chloroquine-enhancing activity of these analogues of strychnobrasiline.

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RESULTS AND DISCUSSION

C_{20}-C_{21} double bond in strychnobrasiline analogues was previously reported to be particularly unreactive towards common reductive reagents.[3] Our program aimed at the transformation of **1** into **2** required a better knowledge of the ability of **1** to be chemically modified. Therefore we decided to re-examine the outcome of **1** when submitted to catalytic hydrogenation and to investigate its reactivity towards $LiAlH_4$ reagents.

Catalytic hydrogenation

Hydrogenation of **1** with 10% Pd on charcoal in 1N HCl did not reduce the double bond producing instead hydrogenolysis of the ether linkage. After 2 hours at room temperature, **3** was isolated in 30% yield. The most relevant difference in the ^1H NMR spectra was the disappearance of the doublet attributed to CH_3-18 (δ 1.31, J=6) in **1** and the consequent presence of a triplet at higher field (δ 0.99, J=7.4). When aged for 24h the reaction led to the derivative **4** (80% yield) in which the N-acetyl group has been hydrolysed.

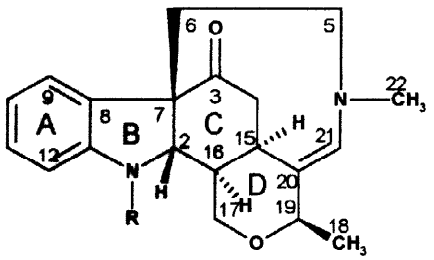
Structures of **3** and **4** were routinely deduced from mass, ^1H and ^{13}C NMR studies compared to strychnobrasiline[4] and N-desacetyl strychnobrasiline[5], **1a**, spectral data. Similar reaction has been previously described from 10-11,dimethoxystrychnobrasiline[3].

Lithium aluminium hydride reduction

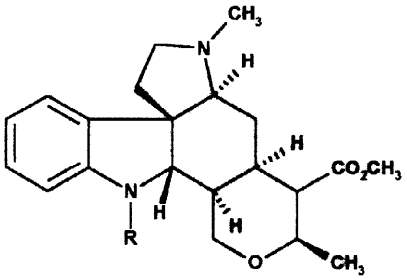
In first experiment, **1** was dissolved in anhydrous THF and treated by large excess of $AlLiH_4$, used as pellet. $AlLiH_4$ was totally dissolved after 1h and the mixture was further stirred for 1h at room temperature. After treatment by water saturated ethyl ether, a complex mixture of six compounds was obtained and flash chromatographed. Using successively CH_2Cl_2-MeOH (98:2), (95:5), CH_2Cl_2-MeOH-NH_4OH (90:10:1) and (85:15:2) as eluent, compounds **5** (3%) and **6** (3%), **7** (40%) and **8** (10%), **9** (10%) and finally **10** (25%) were separated. In an other experiment, the reaction was carried out using $AlLiH_4$ dissolved in anhydrous THF. The hydride solution was added dropwise during five minutes and the reaction maintained at room temperature for 2h. After usual work up, compound **10** was isolated in high yield (72%).

The structures of the new products were determined using spectral measurements, mainly NMR 1D and 2D techniques. From preliminary data, it clearly appeared that **5** and **6**, **7** and **8**, **9** and **10** respectively differed from each other with respect to substitution of the indoline nitrogen atom. The initial N-acetyl group present in **1** was hydrogenolyzed in **6**, **8** and **10** and reduced to N-ethyl in **5**, **7** and **9**.

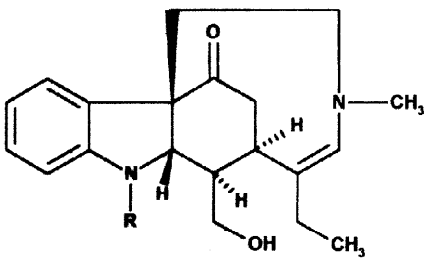
Compound 6. The molecular formula of **6** ($C_{20}H_{26}N_2O_2$) was deduced from the mass spectra data. ^1H and ^{13}C NMR spectra showed features similar to those observed for strychnobrasiline. So, the four aromatic protons and the aliphatic protons of tryptamine and tetrahydropyran ring were routinely identified. The most notable difference with **1** was the disappearance of H-21 singlet and the presence of two signals at 4.55 (d, J=7.7) and 2.10 (m) corresponding to H-21 and H-20 coupling protons. Disappearance of C=O in the IR spectrum and deshielding of N_b-CH_3 singlet (δ 2.64) suggested the formation of an ether linkage between C_3 and C_{21}. Structure was definitively confirmed from 2D NMR data : ^1H-^{13}C long range correlation's from the HMBC spectrum allowed the assignment of signals at 89.0 ppm to C-21, which correlated with H-3 (δ 4.01, bt), H-19 (δ 3.43,dq) and 75.9 ppm to C-3 correlated with H-2 (δ 4.05,d) and H-21 (δ 4.55,d). Further correlations were observed between H-21 and C-22 (δ 45.1) and C-5 (δ 47.5).



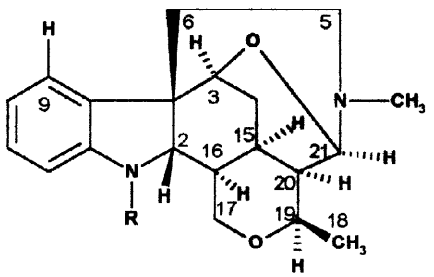
1: R=COCH$_3$
1a: R=H



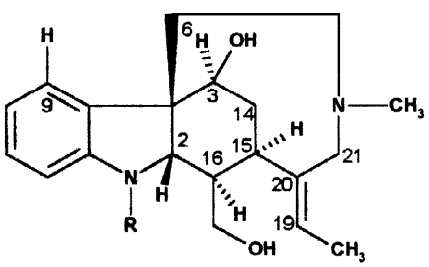
2: R=COCH$_3$



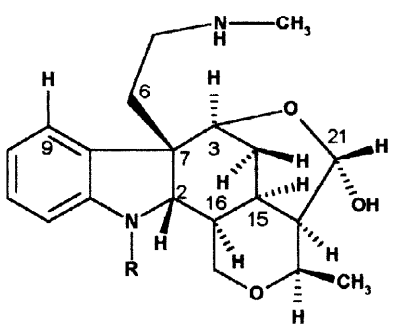
3: R=COCH$_3$
4: R=H



5: R = C$_2$H$_5$
6: R = H



7: R = C$_2$H$_5$
8: R = H



9: R = C$_2$H$_5$
10: R = H

Fig 1. Structure of strychnobrasiline **1**, malagashanine **2**, and new compounds.

NOESY spectrum allowed to determinate the relative configurations of **6**. Cross peaks between H-2 and H-5, H-6b defined B-C rings cis junction. NOEs between H-15 and H-16, H-19 indicated chair conformation of the tetrahydropyran ring with C-D rings cis junction, H-15, H-17b and H-19 being axial and then CH_3-18 equatorial. NOESY relationship CH_3-H_{21}, H-20 indicated α position for H-21 and then R configuration was attributed to C-21. Lastly NOE between H-3 and H-9, revealing the steric proximity of H-3 and aromatic ring, established the relative configuration of C-3.

Compound 7. The structure of **7** was easily deduced from the spectral data. Mass spectrum provided the molecular formula $C_{22}H_{32}N_2O_2$. In the IR spectrum C=O absorption band was lacking and the NMR data showed opening of the tetrahydropyran ring. C_{19}-C_{20} double bond was evidenced by the fact that the singlet at 5.95 was replaced by a doublet at δ 1.67 (J=7.3) and quadruplet at 5.47 (J=6.6) attributed to CH_3-18 and H-19 respectively. The complete assignments of ^1H and ^{13}C spectra have been done with the help of 2D NMR data. From the HMBC spectrum, C_3 (δ 69.4) appeared to correlate with H_2, H_6 and H_{15}; H-3 (δ 3.76,m) was identified by the observation of cross peaks with C-2, C-6 and C-14. The C-3 configuration was supported by the presence of a cross peak between H-3 and H-9 in the NOESY spectrum.

Compound 10. Molecular formula of **10** ($C_{20}H_{28}N_2O_3$) was deduced from the mass spectra analysis. **9** is likely to possess a hydroxyl group on account of the peak at m/z 326 (M-18) and the indoline-β-CH_2-CH_2-N-CH_3 on account of peaks at m/z 144, 143 and m/z 284 (M-59). The analysis of ^1H and ^{13}C data showed that its structure was closely related to **6**: the tetrahydropyran ring and the the tryptamine moiety were unchanged. The important and distinct features were the cleavage of the C-21-NCH_3 bond and the hemiacetal formation which were evidenced from ^1H NMR spectra (DMSO) : two signals at δ 5.90 and δ 6.55 were assigned to exchangeable protons, the first one to indolinic NH and the second, which from the COSY spectrum data correlated with H-21, to hydroxylic proton. Furthermore from the chlorohydrate spectrum triplet at δ 2.42 (J=5.2 Hz) was attributed to N_bCH3. This assignment was substantiated by correlation observed between C-22 and signals attributed to protonated amine at δ 8.85 and δ 8.99.

As in the case of **6**, relative configurations assignment was attributed from NOESY spectrum. Similar conclusions were obtained : H_2-C_6 and H_{15}-H_{16} cis positions were defined and chair conformation with equatorial CH_3-18 attributed to the pyran ring. NOE between H-3 and H-9 clearly defined α position of H-3. The distinct feature was the β position of H-21 which was deduced from NOEs observed between H-21 and H_2, H_6. Then C-21 has S relative configuration.

During this work, we observed that treatment of strychnobrasiline **1** by $LiAlH_4$ gave rise to unexpected derivatives. Behaviour of **1** may be explained in light of peculiar structure of Nb-methyl-sec-pseudo series of strychnos alkaloids deduced from ^1H and ^{13}C NMR spectra .[4] H-21 olefinic proton is deshielded and appeared as a singlet at δ 6ppm. This is explained by an intramolecular exchange interaction between the double bond and the C-3 carbonyl. Furthermore in ^{13}C spectrum, the C-3 carbonyl signal is greatly shielded (δ 192.5 ppm) due to a non bonded interaction between Nb and C_3. These structural features[3] explain the non reactivity of the C-3 carbonyl towards $NaBH_4$ and the greatly diminished reactivity of the C-20, C-21 double bond which does not undergo typical reactions of neostrychnine double bond. In this work we showed that Pd on charcoal in HCl did not reduce the olefinic bond but produce hydrogenolysis of the ether linkage to give **3**.

In other way, trivalent aluminium atom is known to be capable of coordinating with unshared electron pair[6]. When **1** was treated by $LiAlH_4$, pyran reactivity should be favoured and formation of an alkoxy aluminium complex might occur preferentially. Therefore, after reversible pyran ring opening and migration of C-20, C-21 double bond, next step should be the formation of conjugate immonium. This intermediate could either be reduced to give **7** or react with alkoxy aluminium complex at C-3.

Biological evaluation

Products **2** to **10** were tested comparatively to strychnobrasiline **1**, *in vitro* on the chloroquine resistant strain FcB1 of *Plasmodium falciparum*, the main causative agent of malaria, for their ability to potentiate the chloroquine inhibitory effect. Drug interactions were estimated by measuring the chloroquine IC_{50} in the presence of fixed concentrations of the tested product (lower than its own IC_{50}), and isobolograms of interaction constructed. An interaction factor (IF) was defined[7] as the reciprocal of the chloroquine fractional IC_{50} obtained from the experimental curve for half of the IC_{50} of each product tested. The IF equals 2, <2, or >2 for additive, antagonistic, or synergistic effect on chloroquine inhibition. For synergistic effect, the higher IF values, the higher its potentiating activity on chloroquine.

Table 1 : *In vitro* potentiating action (IF) against the chloroquine resistant *Plasmodium falciparum* strain FcB1

Product	1	1a	3	4	5	6	7	8	10
	10.0	37.0	25.0	22.2	2.1	3.6	29.0	5.5	2

From the results reported in Table 1, all the molecules tested potentiate the activity of chloroquine, except compound **10**, which presents an additive activity. N-deacetyl strychnobrasiline, **1a**, was found twice more potent than strychnobrasiline **1**. These data suggest that the Nb,C(21)-secocuran skeleton is necessary to the synergistic activity which is significantly influenced by the basicity of the indolinic nitrogen atom. Work is in progress to prepare novel analogues of strychnobrasiline to establish structure activity relationship.

EXPERIMENTAL

General experimental procedures : Optical rotations were measured with a Perkin Elmer 241 polarimeter and the IR spectra on a Nicolet Impact 400D interferometer. Mass spectra were recorded on a Nermag R10-10 apparatus, while high-resolution data were measured on a Jeol MS700. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300.13 and 75.47 MHz respectively on a Bruker AC-300 spectrometer by using standard program. The ^1H and ^{13}C chemical shifts are expressed in ppm from TMS. Coupling constant (J) are given in Hz. 2D NMR NOESY, HMQC and HMBC experiments were carried out on a Bruker AM 400 spectrometer using a triple resonance probe head with gradient selection of coherence transfer pathway.

Column chromatography were carried out on 200-400 mesh silica gel 60(Merck). Melting points (mp) were taken on a Reichert hot stage microscope and are uncorrected.

Hydrogenation of strychnobrasiline

To a solution of 100 mg of strychnobrasiline, **1**, in 3 ml 1N HCl, 50 mg of 10% Pd/C were added and the mixture hydrogenated for 1 h at room temperature under atmospheric pressure. After filtration of the catalyst, the solution was neutralized and extracted by CH_2Cl_2. The solvent was evaporated under vacuum and the residue obtained chromatographed on silica gel. Elution with the mixture CH_2Cl_2-MeOH-NH_4OH (95/5/0.5) afforded besides starting material 30mg of **3**. When the reaction was monitored for 24h work-up of the solution obtained after filtration gave 90mg of **4**.

Compound 3 :

IR: max (film) : 3413, 3310,1655, 1474, 1066, 1010 cm^{-1}.

^{1}H NMR: δ 0.99 (t, 7.4, CH_3-18), 1.84 (m, 1H), 2.0 (m, 3H), 2.06 (s, N-CH_3), 2.27 (s, $COCH_3$), 2.37 (m, 2H), 2.63 (bs, 1H), 2.76(m, 2H), 3.01 (m, 1H) 3.56, (d, 10.9, H-2), 4.14 (m, CH_2-17, H-19), 5.88(s, H-21), 6.56 (d, 7.8, H-12), 6.69 (m, H-10), 7.02 (m, H-11), 7.51 (d, 7.4, H-9).

^{13}C NMR : δ 13.0 (C-18), 20.8 (C-24), 28.8(C-19), 35.9 (C-15), 40.8 (C-6), 41.0 (C-14), 42.0 (C-22), 43.7 (C-16), 55.4 (C-5), 57.8 (C-7), 61.8 (C-2), 66.4 (C-17),109.4 (C-12), 119.1 (C-10), 126.9 (C-9),128.3 (C-15), 129.4 (C-8), 136.6(C-21), 136.6 (C-20),148.5 (C-13), 170.6 (C-23).

EIMS m/z = 368 (66), 352 (18), 340 (12), 170 (36), 144(100), 124 (45), 110 (75), 85 (70).

HRMS for $C_{22}H_{28}O_3N_2$: $M^{+\cdot}$, calc. 368.2100, found 368.2094.

Compound 4 : mp = 201-202°C (acetone),$[\alpha]_D$= +145° ($CHCl_3$, c=0.94).

IR ν_{max} (KBr): 3397, 3316, 1635, 1470, 1414, 1380, 1250, 1066, 1010 cm^{-1}.

^{1}H NMR: 0.94 (t, 7.4, CH_3-18), 1.82 (m, H-19b), 1.88 (m, H-16b), 1.95 (m, H-16a), 2.00 (s,N-CH_3), 2.01 (m, H-19a), 2.31 (bt, CH_2-14), 2.48 (m, H-5b, H-15), 2.86 (m, H-5a-H-6a), 3.76 (d, 10.8, H-2), 3.82(m, CH_2-17), 5.80 (s, H-21), 6.61 (d, 7.7, H-12), 6.74(ddd, 0.6, 7.33, H-10), 7.02 (ddd, 1.2, 7.5, H-11), 7.46 (d, 7.4, H-9).

^{13}C NMR : 13.4 (C-18), 29.1 (C-19), 37.4(C-15), 42.0 (C-22), 42.4 (C-6), 43.8 (C-16), 44.1 (C-14), 53.3 (C-5), 58.0(C-17), 64.1 (C-2), 67.9 (C-17), 110.6 (C-12), 119.5 (C-10), 125.6 (C-9), 128.1(C-11),131.3 (C-8), 137.5 (C-21), 139.2 (C-20), 148.6 (C-13),190.4 (C-3), 190.4 (C-3).

EIMS m/z = 326 (100), 298 (19), 267 (31), 187 (31), 170(18), 144 (73), 110 (62), 84 (46).

HRMS for $C_{20}H_{26}N_2O_2$ = $M^{+\cdot}$, calc. 326.1994, found 326.1988.

Lithium aluminium hydride reduction of strychnobrasiline

To a solution of strychnobrasiline (200mg) in anhydrous THF (10ml) was added $LiAlH_4$ as pellets (160mg). The suspension was stirred at room temperature for 2h and then treated by water saturated ether. After filtration, the precipitate was washed successively with ether and methanol. The solvents were evaporated and the resulting residue dissolved in CH_2Cl_2. After washing and evaporation the residue obtained was chromatographed on silica gel column. Elution with CH_2Cl_2-MeOH (98:2), (95:5), CH_2Cl_2-MeOH, NH_4OH (90:10:1) and (85-15-2) gave successively **5** (7mg) and **6** (7mg), **7** (81mg) and **8** (20mg), **9** (19mg) and **10** (50mg).

Compound 5 :

IR$\lambda\nu_{max}$ (film) = 3384, 1604, 1480, 1100 cm^{-1}.

^{1}H NMR: δ1.03 (t, 7.0, CH_3-24),1.20 (m, H-6b), 1.44 (d, 6.7, CH3-18),1.63 (m,H-14b), 1.78 (m, H-14a),1.98 (bt, 5.5, H-16), 2.14 (m, H-20), 2.24 (m, H-15), 2.51 (dd, 15.5, 4.7,H-5b), 2.67 (s, CH_3-22), 3.00 (m, H-23a, H-6a), 3.31 (m, H-23b), 3.45 (dq, 2.8, 6.7, H-19), 3.89(dd, 12.9, 2.5, H-5a), 3.97 (dd, 12.0, 4.9, H-17b), 4.13 (d, 12.0, H-17a),4.59 (d, 9.3, H-21), 6.40 (d, 7.2, H-12), 6.66 (t, 7.4, H-10), 7.03 (t, 7.7, H-11) 7.08 (d, 6.3, H-9).

^{13}C NMR : δ 9.4 (CH_3-24), 18.3 (C-19), 28.9 (C-15), 30.2 (C-14), 37.9 (C-6), 38.0 (C-20), 39.4(C-23), 39.6 (C-16), 45.0 (C-22), 46.8 (C-5), 53.2 (C-7), 68.5 (C-2), 74.4(C-17), 76.4 (C-3), 76.8 (C-18), 88.9 (C-21), 106.8 (C-12), 117.8 (C-10), 122.9 (C-9), 127.8 (C-11), 138.1 (C-8), 148.4 (C-13).

EIMS m/z = 354 (78), 296 (90), 196 (100), 184 (41), 171(35), 158 (90), 143 (25), 144 (25), 130 (41), 115 (25).

HRMS for $C_{22}H_{30}O_2N_2$: $M^{+\cdot}$, calc. 354.2307, found 354.2305.

Compound 6 :

IR: ν_{max} (film) : 3370, 1600, 1485, 1375, 1127cm^{-1}.

^{1}H, ^{13}C NMR (see Table 2).

EIMS : m/z = 326 (55), 283 (14), 268 (55), 196 (32), 185(38), 168 (100), 156 (52), 144 (50), 143 (65), 130 (57).

HRMS for $C_{20}H_{26}N_2O_2$: $M^{+\cdot}$, calc. 326.1994, found 326.1995.

Table 2 : ^{1}H and ^{13}C NMR chemical shifts (ppm-), multiplicity, coupling constants (Hz) and NOEs of compounds **6**, **10** ($CDCl_3$).

	6			**10**		
C	**δ_C**	**δ_H, J(Hz)**	**NOEs**	**δ_C**	**δ_H,M, J(Hz)**	**NOEs**
2	65.8	4.05, d, 2.2	5, 6b	69.9	3.48, d, 7.2	5.6, 12, 17a, 21
3	75.9	4.01, bt, 2.6	6, 9, 14a, 14b	71.8	4.36, d, 3.4	5.9, 14a, 14b
5 a	47.5	3.69, m	5b, 6b	47.4	2.68, m	6, 9
5 b		2.59, m	6a, 6b		2.55, m	
6 a	36.8	2.88, m	6b, 9	36.9	1.85, m	21, 22
6 b		1.34, ddd,13.7, 5.9, 3.1				
7	53.8			52.2		
8	137.6			137.6		
9	123.1	7.08, dd, 7.4, 0.8	10	124.2	6.95, d, 7.7	14
10	118.9	6.7, ddd, 7.5, 7.4, 1	11	119.5	6.70, dd, 7.7, 7.2	
11	127.6	6.98, ddd, 7.4, 7.5, 1	12	129.2	7.02, dd, 7.9, 7.2	
12	108.4	6.48, d, 7.7		111.9	6.65, d, 7.9	
13	147.8			150.8		
14 a	30.2	1.80, m	14b, 15	31.2	1.83, m	14b, 15, 20
14 b		1.72, m	15		1.50, m	15
15	28.8	2.20, m	16, 19, 20	32.6	1.92, m	16, 19,20
16	40.5	1.96, m	17b	42.8	1.32, m	20
17 a	73.6	4.18, d, 11.2	17b	72.3	3.97, d, 11.6	16, 17b
17 b		3.89, dd, 12.2, 4.9	19		3.56, dd, 11.6, 2.2	
18	18.5	1.42, d, 6.7	19, 20, 21	20.6	1.27, d, 6.5	19, 21, OH
19	76.7	3.43, dq, 2.9, 6.7	20	76.7	3.48, m	20
20	40.0	2.10, m	21	46.7	1.49, m	OH
21	89.0	4.55, d,7.7	22	92.5	5.07, d, 8.5	OH
22	45.1	2.64, s		35.5	2.33, s	

Compound 7 :

IR ν_{max} (film) : 3384, 1606, 1488, 1414, 1383, 1265, 1035 cm^{-1}.

^{13}C NMR : δ 10.6 (C-24), 13.6 (C-18), 29.8 (C-14), 27.9 (C-6), 41.9 (C-23), 42.7 (C-16), 43.6 (C-15), 45.8 (C-22), 47.1 (C-21), 48.0 (C-7), 53.7 (C-5), 65.1 (C-2), 66.5 (C-17), 69.4 (C-3), 106.5 (C-12), 117.0 (C-10), 123.9 (C-9), 124.4 (C-19), 128.9 (C-11), 135.9 (C-8), 140.3 (C-20), 150.9 (C-13).

^{1}H NMR : δ 1.09 (t, 6.8, CH_3-24), 1.65 (m, H-14b), 1.67 (d, 7.3, CH_3-18), 1.88 (m, H-6b), 2.22 (s, CH_3-22), 2.27 (m, H-14a, H-16), 2.54 (m, H-5b, H-6a), 2.74 (m, H-5a), 2.89 (m, H-15), 3.12 (d, 5.3, H-21b), 3.24 (m, H-23b), 3.40 (m, H-21a, H-23a), 3.76 (m, H-3, H-17b), 3.86 (d, 11.1, H-17a), 4.02 (d, 7.8, H-2), 5.47 (q, 6.6, H-19), 6.41 (d, 7.7, H-12), 6.70 (t, 7.3, H-10), 7.09 (d, 7.5, H-9), 7.09 (t, 7.5, H-11).

EIMS : m/z = 356 (19), 249 (32), 198 (100), 172 (84), 158(63), 144 (31), 130 (51).

HRMS for $C_{22}H_{32}N_2O_2$: $M^{+}\cdot$, calc. 356.2464, found 356.2469.

Compound 8 : mp=230°-232°C (acetone), $[\alpha]_D$= +70° ($CHCl_3$, c=0.35).

IR ν_{max} (KBr) : 3372, 1600, 1485, 1374, 1127, 1100, 1065, 947 cm-1.

^{1}H NMR : 1.61 (d, 6.61,CH_3-18), 1.67 (m, H-14b), 1.85 (bd, 12.4, H-6b), 2.23 (s, N-CH_3), 2.22 (m, H-14a, H-16), 2.55 (m,CH_2-5, H-6a), 2.83 (m, H-15), 3.0 (bd, 15.9, H-21b), 3.34 (bd, 14.8, H-21a), 3.59 (dd, 10.5, 6.9, H-17b), 3.71 (dd, 10.6, 5, H-3), 3.84 (dd, 11.6, 1,H-17), 4.24 (d, 7.8, H-2) 5.32 (q, 6.7, H-19), 6.51 (d, 7.8, H-12), 6.68 (dd, 8.3, 0.9, H-10), 7.01 (dd, 8, 1.2, H-11), 7.10 (d, 7.2, H-9).

^{13}C NMR: δ 13.4 (C-18), 28.4 (C-6), 30.2(C-14), 42.3 (C-16), 44.4 (C-15), 44.8 (N-CH_3), 46.6 (C-16), 48.9 (C-7), 53.1 (C-5), 65.4 (C-17), 65.8 (C-2), 69.6(C-3), 108.4 (C-12), 118.0 (C-10), 123.6 (C-9), 124.2 (C-19), 128.0 (C-11), 136 (C-8), 139.9 (C-20), 150.2 (C-13).

EIMS: m/z = 328. (23), 221 (93), 198 (71), 181 (47), 168(81), 144 (87), 130 (100), 83 (77).

HRMS for $C_{20}H_{28}N_2O_2$: $M^{+\cdot}$, calc. 328.2151,found 328.2153.

Compound 9

IR ν_{max} (KBr): 3372, 1600, 1485, 1374, 1127, 1100, 1065, 947 cm^{-1}.

^{1}H NMR (CD3OD) : 1.27 (t, 7, CH3-24), 1.30 (d, 6.8,CH3-19), 1.51 (m, 2H) 1.83 (m,1H), 1.97 (m,3H), 2.60 (s,CH3-22), 2.92 (m,H-5a,b), 3.48 (d,7.1, H-2), 3.59 (dd, 11.8,3.3,H-17b), 4.10 (d, 11.7, H-17a), 4.37 (d, 4, H-3), 5.03 (d, 9, H-21), 6.45 (d, 7.9, H-12), 6.60(t,7.2,H-10), 6.95 (d, 7.3, H-9), 7.07 (t,7.07, H-11).

^{13}C NMR: δ 13.6 (C-24), 20.5 (C-18), 30.8 (C-14), 33.0(C-22), 33.6 (C-15), 34.8 (C-6), 40.3 (C-23), 42.6 (C16), 45.9 (C20), 46.6 (C-5), 51.3 (C-7), 69.9 (C-2), 71.3 (C3), 73.2 (C17), 78.1(C-19), 92.5 (C-21), 107.9 (C-12), 117.8 (C-10), 123.8 (C-9), 130.0 (C-11), 131.7 (C-8), 149.5 (C-13).

EIMS: m/z = 372 (3), 346 (3), 287 (31), 270 (69), 194(20), 168 (44), 156(37), 144(83), 130 (82), 109 (35), 91(40), 71 (62),59 (79), 44 (100).

HRMS for $C_{22}H_{33}N_2O_3$ (M^{+1}) : calc. 373.2491, found 373.2485.

Compound 10 was obtained as the major product using the following procedure: to a solution of 367 mg of strychnobrasiline in anhydrous THF (12ml) was added via syring 2 ml of 1M $LiAlH_4$ solution in THF. The mixture was stirred under argon at room temperature for 1h. A mixture of ether/H_2O, (95/5) was added and the precipitate was filtered and washed with 5 ml of methanol. The filtrate was concentrated in vacuo and chromatographed on silica gel. Elution with CH_2Cl_2-MeOH-NH_4OH (85/15/2) gave **10** (250 mg, yield 72%); mp > 250° (dec) (MeOH); $[\alpha]_D$ = -26° (MeOH, c = 0.35).

IR max (KBr) : 3484, 3342, 1693, 1476, 1389,1128,1080,1022, 979, 948cm^{-1}.

^{1}H, ^{13}C NMR: (see Table 2).

EIMS : m/z= 344 (30), 285 (100), 286 (130), 269 (14), 268(1), 144 (37), 143 (32). **ICMS** (isobutane): **m/z**= 345 (100), 327 (18).

HRMS for $C_{20}H_{28}N_2O_3$: $M^{+\cdot}$, calc. 344.2100, found 344.2111.

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