Synthesis of antileishmanial (*5R*)-(-)-5-carbomethoxy-3-formyl-5,6-dihydroindolo-[2,3-*a*]-indolizine

Parasuraman Jaisankar[a], Bikash Pal[a], Ranjan K. Manna[a], Prasun K. Pradhan[a], S. Medda[b], Mukul K. Basu[b] , and Venkatachalam S. Giri[a]*

[a]Department of Medicinal Chemistry, [b]Biomembrane Division,
Indian Institute of Chemical Biology,
4, Raja S. C. Mullick Road, Jadavpur, Calcutta – 700 032, India
E-mail: vsgiri@iicb.res.in

Dedicated to Professor (Mrs.) A. Chatterjee on her 85th anniversary
(received 24 Oct 03; accepted 16 March 04; published on the web 19 March 04)

Abstract

Synthesis of 5-carbomethoxy-3-formyl-5,6-dihydroindolo-[2,3-*a*]indolizines (**10** and **12**) and 6-carbomethoxy-3-hydroxy-6,7-dihydroindolo[2,3-*a*]quinolizinium sulphates (**11** and **13**) have been achieved. Interestingly, the compound (5*R*)-(-)-5-carbomethoxy-3-formyl-5,6-dihydroindolo[2,3-*a*]indolizine (**10**) intercalated mannose-grafted microspheres was found to be an effective drug, whereas its optical antipode **12** showed no activity at all, against parasite, *Leishmania donovani* strain AG83 in hamsters model both *in-vitro* and *in-vivo*.

Keywords: Chiral β-carbolines, dihydroindoloindolizine, dihydroindoloquinolizine, manose-grafted micro spheres, anti-leishmanial agent, *Leishmania donovan*i

Introduction

The syntheses of optically active indole alkaloids have continued to attract synthetic organic chemists because of their physiological importance. It has recently been reported[1] that antiviral properties in a few indole alkaloids are due to the presence of the indolizine ring system. In our endeavour to synthesis a few of these optically active indole alkaloids having the indolizine ring system, two approaches have been utilized involving the famous Pictet-Spengler condensation[2] between tryptamine (**1**) or tryptamine methyl ester and a sugar derivative **4**. In one approach, sugar itself could be expected to transfer its chirality in the course of generating the β-carboline product and in the other approach, D- or L-tryptophan methyl ester (**2** or **3**) was the choice for obtaining the desired chiral product.

The term Leishmaniasis comprises of broad spectrum of diseases caused by different species of kinetoplastid protozoa belonging to the genus *Leishmania*[3]. The visceral Leishmaniasis or kala-azar is transmitted by female sand flies of the species *Phlebotomus*, which causes death with systematic damage of soft tissues of human body. Three million individuals suffer from various forms of Leishmaniasis, the number of new cases each year being 1.5 milllion of which 5,00,000 are visceral leishmanisis. Although there are quite a number of drugs used for treatment of this disease, including the famous pentavalent antimonial drug sodium stibogluconate, all of them cause serious side effects. We report herein the synthesis of a potent anti-leishmanial indolo-[2,3-*a*]-indolizine **10**.

Results and Discussion

Earlier we have reported[4] the condensation of tryptamine (**1**) with the anhydrosugar **4** in aqueous methanolic solution with NaOAc-HOAc buffer resulting in the chiral[5] β-carboline **5**. The next step was the crucial acid hydrolysis involving deketalisation followed by cyclisation leading to the cyclised products. Compound **5** on hydrolysis with 4% H_2SO_4 in acetonitrile-water mixture at room temperature gave **8** and **9** as minor and major product respectively. On the other hand, when hydrolysis was performed with 2.5% H_2SO_4 in acetic acid-water mixture at 60°C, the compounds **8** and **9** were formed in almost equal proportions. The observation here was that acid treatment of compound **5** resulted in loss of chirality due to dehydration following cyclisation to either a indoloquinolizine or indoloindolizine ring system.

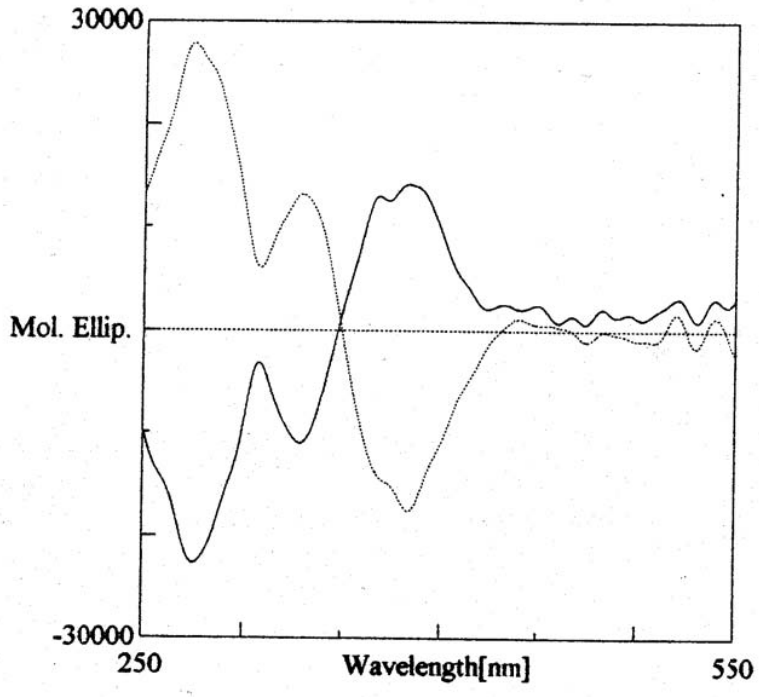
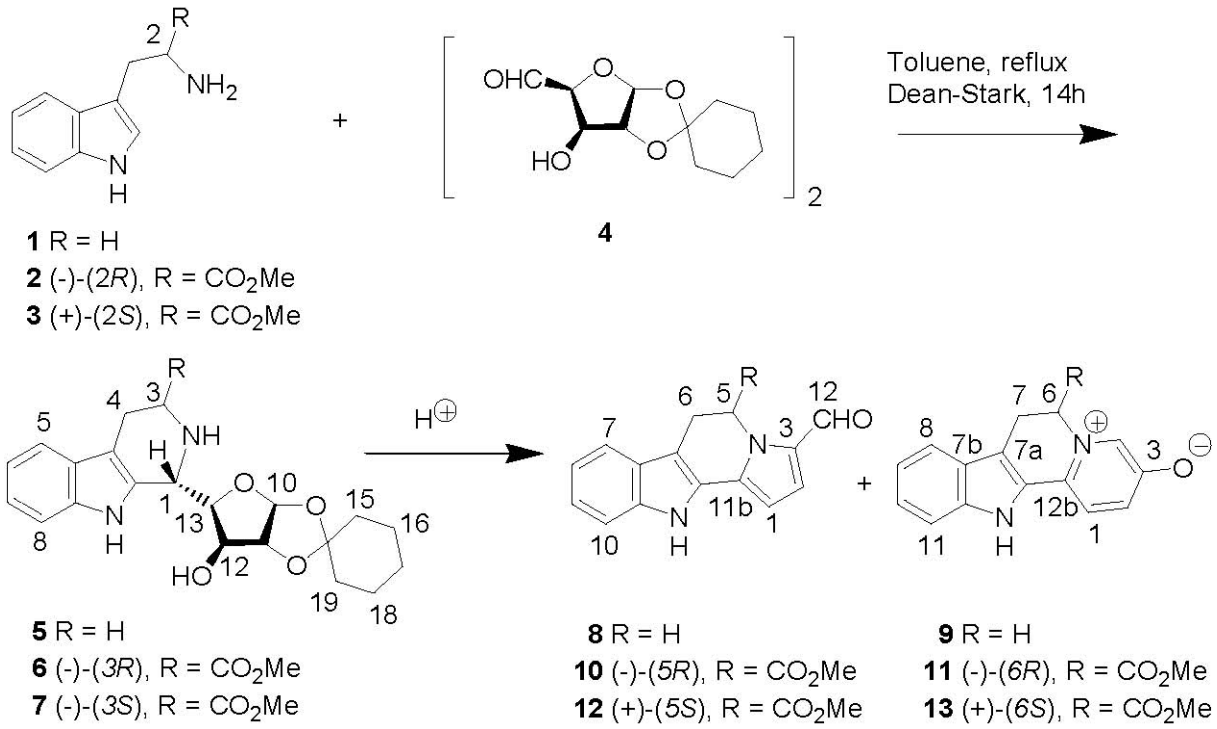


Figure 1. CD Spectra of Compounds **10** (------) and **12** (——).

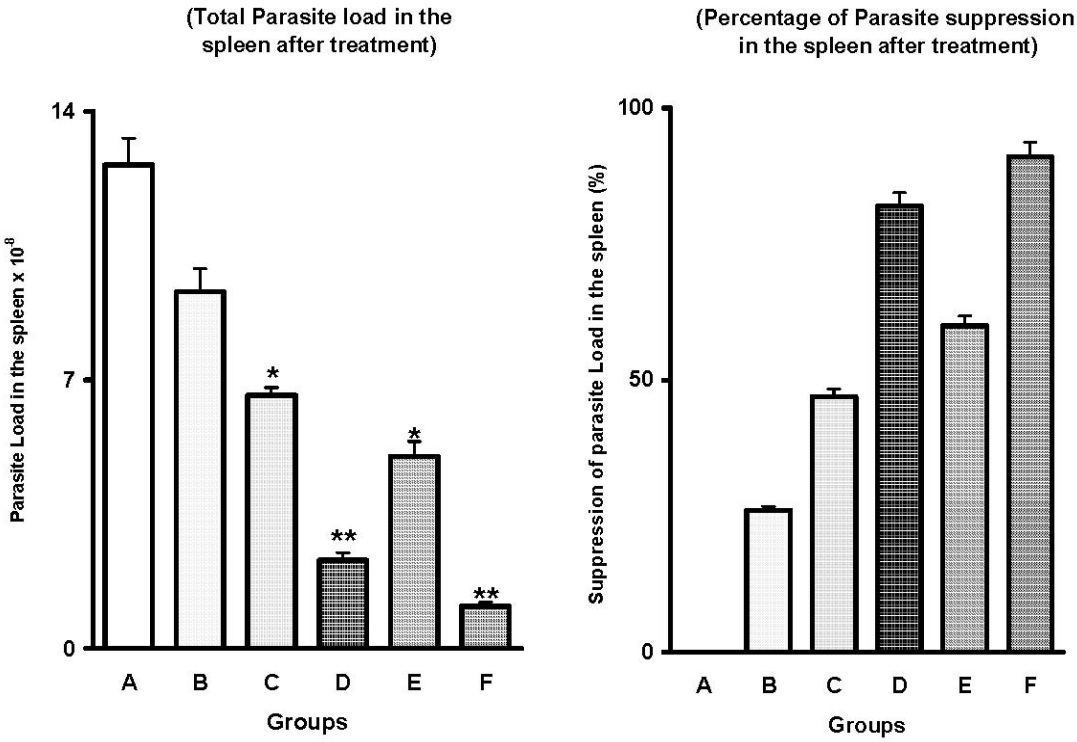
The condensation of D-tryptophan methyl ester (**2**) with the anhydrosugar **4** afforded the chiral β-carboline **6** whereas the L-isomer **3** with the same sugar **4** under the same conditions afforded the diastereoisomer **7**. Hydrolysis of the compound **6** with 4% H_2SO_4 in acetonitrile-water mixture gave the aldehyde **10** characterised as (5R)-(-)-6-carbomethoxy-3-formyl-5,6-dihydroindolo[2,3-*a*]indolizine along with the quarternary salt, (6R)-(+)-6-carbomethoxy-3-hydroxy-6,7-dihydroindolo[2,3-*a*]quinolizinium hydrogen sulphate (**11**) (scheme 1). As expected, the acid hydrolysis of the other diastereoisomer **7** with 4% H_2SO_4 in acetonitrile-water mixture gave the corresponding isomers (5S)-(+)-isomer **12** and (6S)-(-)- isomer **13** respectively. We believe that the mechanism proposed earlier by us[4] for the formation of **8** and **9** holds good for the corresponding tryptophan methyl ester also. The salts **11** and **13** were found to be rather labile. They had the tendency to decompose along with racemisation (particularly compound **13**), whereas the chiral aldehydes **10** and **12** were comparatively quite stable and the CD spectra (Figure 1) of the aldehydes conclusively establish this fact. All the new compounds have been characterized mainly from their NMR, IR, MS, and CD data.



Scheme 1

Leishmanicidal activities

The leishmanicidal activity of (5R)-(-)-6-carbomethoxy-3-formyl-5,6-dihydroindolo[2,3-*a*]-indolizine (**10**) was done viz, free, liposome-intercalated, microsphere-intercalated, mannose bearing liposome-intercalated and mannose bearing microsphere-intercalated against infected hamsters model of *L. donovani*[6] and the results are shown in Figure 2.



Groups A = Infected + untreated; B = Infected + free dug; C = Infected + liposomal drug; D = Infected + mannose bearing liposomal drug; E = Infected + microsphere intercalated drug; F = Infected + mannose bearing microsphere intercalated drug.
The value are expressed as mean $\pm$ SD (n = 4).
Dose given to each animal each time was 3 mg/kg body weight.
Percent suppression of parasite load using empty vesicles was found to be in the range of 14-18%.
*p <0.003 compared to infected + untreated.
**p <0.001 compared to infected + untreated.

Figure 2. Effect of (5*R*)-(-)-6-carbomethoxy-3-formyl-5,6-dihydroindolo[2,3-*a*]-indolizine (**10**) on 30 days infected hamsters model of *Leishmania donovani*.

Subcutaneous injection of free dihydroindolo-[2,3-*a*]-indolizine **10** (3 mg/kg body weight) reduced the parasite load by 26%, whereas the intercalation of the drug in the liposomes and microspheres reduced the parasite burden to 47 and 60% at the same micro-viscosity level. The efficacies in terms of reduction of parasite load varied from 91% in mannose coated liposomes (Figure 2). It is very interesting to note that the optical antipode **12** of the compound **10** showed no activity at all, where as 3-formyl-5,6-dihydroindolo-[2,3-*a*]indolizine (**8**) showed moderate activity against *Leishmania donovanai*. This suggests that enantiomerically pure compound **10** may selectively bind the active site of some vital enzymes thereby inhibiting the growth of *L. donovani* needed for their survival or proliferation. However the efficacy of the drug **10** against

experimental leishmania is increased if the drug is used in the liposome incorporated or microsphere incorporated form. The efficacy of the drug **10** is further increased to 91% when the mannose-bearing liposomal form or mannose bearing microsphere-incorporated form. It is apparent that because of longer arms, the mannose sugar attached to microsphere and liposomes may be more accessible to specific receptors on macrophages[7]. The toxicity studies on normal liver for specific enzyme levels in sera; kidney for urea level creatinine levels showed no apparent toxicity.

To summarize, we have developed a convenient method for synthesis of a potential anti-leishmanial drug 5-carbomethoxy-3-formyl-5,6-dihydroindolo-[2,3-*a*]indolizines (**10**) and its optical antipode, with no normal toxicity.

Experimental Section

General Procedures. All melting points were determined in open capillaries on SPAC-N-SERVICE (India) capillary melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT-IR Model 410 using samples as KBr plates. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker DPX 300 NMR instrument using TMS as internal standard. Optical rotations were recorded at 25^{o}C in P-1020 JASCO polarimeter. Circular Dicroism (CD) spectra were recorded on a JASCO J-720 spectropolarimeter interfaced with a Compaq PC 486 in rectangular quarts cells of 1 cm path length. UV spectra were recorded on UV-2000 spectrophotometer. Elemental analyses were carried out on a PERKIN ELMER 2400 Series II CHNS/O analyzer. Mass spectra (EI) were recorded on a JEOL AX-500 spectrometer.

10,11-*O*-Cyclohexylidene-12 β-hydroxy-13 β-(3α-carbomethoxy-1-tetrahydro- β-carbolinyl)-tetrahydrofuran (6). A mixture of 2.18 g (10 mmol) of D-Tryptophan methyl ester (**2**) and 2.5 g (5.4 mmol) of the anhydrosugar (**4**) in toluene was refluxed using Dean-Stark apparatus for 14 hrs with continuous removal of water. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed over silica gel. Chloroform eluates were combined, concentrated to afford **6** (3.59 g; 84%) which was recrystallised from petroleum ether (60°C to 80°C)-chloroform. m.p. 175-177°C; $[\alpha]_D^{27.2}$ -60.17° (*c* = 0.339, $CHCl_3$); IR (KBr) : 3364, 2934, 2862, 1740, 1654, 747 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 1.26-1.71 (m, 11H, cyclohexylidene, -OH), 3.11 (dd, *J* = 15.6, 6 Hz, 1H), 3.24 (d, *J* = 15.6 Hz, 1H), 3.62 (s, 3H), 3.87 (d, *J* = 6 Hz, 1H), 4.22 (d, *J* = 3.7 Hz, 1H), 4.42 (t, *J* = 3.9 Hz, 1H), 4.47 (d, *J* = 5 Hz, 1H), 4.65 (m, 4H, NH), 6.13 (d, *J* = 3.7 Hz, 1H), 7.10 (t, *J* = 7 Hz, 1H), 7.18 (t, *J* = 7 Hz, 1H), 7.34 (d, *J* = 8 Hz, 1H), 7.48 (d, *J* = 8 Hz, 1H), 8.16 (brs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) : δ 23.61 (C-18), 23.68 (C-16), 23.89 (C-17), 24.83 (C-4), 36.04 (C-19), 36.79 (C-15), 51.78 (C-1), 51.85 (OMe), 55.64 (C-3), 78.50 (C-12), 81.83 (C-13), 83.62 (C-11), 105.63 (C-10), 105.42 (C-14), 111.13 (C-8), 113.08 (C-4a), 118.16 (C-5), 119.19 (C-6), 122.02 (C-7), 126.76 (C-4b),

131.70 (C-9a), 136.35 (C-8a), 172.37 (C-20); MS *m/z* (rel. int.): 428 (M^+, 17), 427 (59), 369 (26), 271 (48), 230 (100), 211 (20), 183 (62), 170 (90), 169 (100).). Anal. calcd. for $C_{23}H_{28}N_2O_6$: C, 64.47; H, 6.59; N, 6.54. Found: C, 64.90; H, 6.55, N, 6.61.

10,11-*O*-Cyclohexylidene-12β-hydroxy-13β-(3β-carbomethoxy-1-tetrahydro-β-carbolinyl)-tetrahydrofuran (7). A mixture of 2.18 g (10 mmol) of L-Tryptophan methyl ester (**3**) and 2.5 g (5.4 mmol) of the anhydrosugar (**4**) in toluene was refluxed using Dean-Stark apparatus for 14 hrs with continuous removal of water. The reaction mixture was evaporated under reduced pressure and the residue was chromatographed over silica gel. Chloroform eluates were combined, concentrated to afford **7** (3.85 g; 90%) which was recrystallised from petroleum ether (60°C to 80°C)-chloroform. m.p 194°C; $[\alpha]_D^{27.2}$ -77.66° (*c* = 0.394, $CHCl_3$); IR (KBr) : 3446, 3378, 2942, 1742, 1654, 753 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.25-1.75 (m, 11H, cyclohexylidene, -OH), 2.88 (dd, *J* = 11.9, 2.4 Hz, 1H) 3.19 (dd, *J* = 14.4, 2.5 Hz, 1H), 3.77 (dd, *J* = 11.2, 4.1 Hz, 1H), 3.83 (s, 3H), 4.33 (d, *J* = 2.8 Hz, 1H), 4.37 (dd, *J* = 2.8, 6.7 Hz, 1H), 4.55 (d, *J* = 3.6 Hz, 1H), 4.64 (d, *J* = 6.7 Hz, 1H), 4.90 (brs, 1H, NH), 6.11 (d, *J* = 5 Hz, 1H), 7.09 (t, *J* = 7 Hz, 1H), 7.19 (t, *J* = 7 Hz, 1H), 7.50 (d, *J* = 8 Hz, 1H), 7.69 (d, *J* = 8 Hz, 1H), 8.51 (brs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.57 (C-18), 23.88 (C-16), 24.84 (C-17), 25.39 (C-4), 35.56 (C-19), 36.44 (C-15), 52.37 (C-1), 52.60 (OMe), 55.88 (C-3), 75.34 (C-12), 81.76 (C-13), 84.92 (C-11), 104.97 (C-10), 108.26 (C-14), 111.19 (C-8), 112.68 (C-4a), 118.10 (C-5), 119.60 (C-6), 122.10 (C-7), 126.69 (C-4b), 131.67 (C-9a), 136.28 (C-8a), 172.87 (C-20); MS *m/z* (rel. int.): 428 (M^+, 16), 427 (61), 369 (26), 271 (28), 241 (34), 230 (100), 211 (21), 183 (70), 170 (92), 169 (100). Anal. calcd. for $C_{23}H_{28}N_2O_6$: C, 64.47; H, 6.59; N, 6.54. Found: C, 64.81; H, 6.50, N, 6.60.

(5R)-(-)-5-Carbomethoxy-3-formyl-5,6-dihydroindolo[2,3-*a*]indolizine (10). Compound **6** (428 mg, 1 mmol) was stirred in a solution of 0.3 ml conc. H_2SO_4, 1 ml water and 9 ml CH_3CN at room temperature for 72 hrs. The reaction mixture was concentrated, and the residue chromatographed over silica gel. Eluates from petroleum ether (60°C to 80°C)-chloroform mixture (75:25) afforded a solid which was recrystallised from petroleum ether (60°C to 80°C)-chloroform to afford the aldehyde **10** (121 mg; 41%); m.p. 188°C; $[\alpha]_D^{27.2}$ -135.15° (*c* = 0.256, $CHCl_3$); IR (KBr): 3546, 3328, 2952, 1742, 1636, 1608, 756 cm^{-1}; UV (MeOH): λ_{max} (log e) 214.2 (4.48), 269.7 (4.16), 299.6 (3.90), 380.0 (4.37); ^1H NMR (300 MHz, $CDCl_3$): δ 3.45 (dd, *J* = 17 Hz, 7.5 Hz, 1H), 3.61 (s, 3H), 3.75 (dd, *J* = 17 Hz, 1 Hz, 1H), 6.36 (dd, *J* = 7.5 Hz, 1 Hz, 1H), 6.41 (d, *J* = 4 Hz, 1H), 7.03 (d, *J* = 4 Hz, 1H), 7.15 (t, *J* = 7 Hz, 1H), 7.22 (t, *J* = 7 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 7.5 Hz, 1H), 8.34 (brs, 1H, NH), 9.54 (s, -CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.02 (C-6), 52.93 (-CO_2CH_3), 55.73 (C-5), 104.70 (C-1), 107.35 (C-6a), 111.45 (C-10), 118.96 (C-7), 120.49 (C-8), 123.39 (C-9), 125.97 (C-2), 126.14 (C-11b), 126.34 (C-6b), 132.46 (C-11a), 133.09 (C-10a), 137.36 (C-3), 171.39 (-CO_2CH_3), 179.38 (-CHO); MS *m/z* (rel. int.): 294 (M^+, 15), 293 (71), 235 (100), 206 (35); Anal. calcd. for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.30; H, 4.72, N, 9.56.

(6R)-(+)-6-Carbomethoxy-3-hydroxy-6,7-dihydroindolo[2,3-*a*]quinolizinium hydrogen sulphate (11) Further elution of the above column with 10% methanol in chloroform gave a solid which was

recrystallised from methanol to afford **11** (88 mg; 30%). m.p. 280-281°C; $[\alpha]_D^{29}$ +160° (*c* = 0.1, H_2O); IR (KBr) : 3432, 1735, 1626, 745 cm^{-1}3432, 1735, 1626, 745 cm^{-1}; UV (MeOH): λ_{max} (log e) 206.8 (5.24), 223.7 (5.27), 263.9 (4.92), 309.7 (5.15), 321.8 (5.22), 416.0 (4.96); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.51 (m, 1H, H-7), 3.59 (s, 3H, -$COOCH_3$), 3.68 (brd, 1H, H-7), 5.80 (d, *J* = 6.0 Hz, 1H, H-6), 7.05 (d, *J* = 7.5 Hz, 1H, H-11), 7.19 (t, *J* = 7.5 Hz, 1H, H-9), 7.22 (dd, *J* = 8.0 Hz, 2.6 Hz, 1H, H-2), 7.38 (t, *J* = 7.5 Hz, 1H, H-10), 7.56 (d, *J* = 7.5 Hz, 1H, H-8), 7.63 (d, *J* = 2.6 Hz, 1H, H-4), 7.76 (d, *J* = 8.0 Hz, 1H, H-1), 11.81 (brs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.39 (C-7), 53.29 (-CO_2CH_3), 66.59 (C-6), 105.21 (C-7a), 111.69 (C-11), 118.61 (C-8), 119.82 (C-9), 121.53 (C-10), 122.49 (C-12a), 122.88 (C-2), 125.51 (C-7b), 127.42 (C-11a), 134.61 (C-1), 135.65 (C-4), 137.54 (C-12b), 167.3 (C-3), 169.06 (-CO_2Me). FAB-MS *m/z* : 295 $[M]^+$, 317 $[M+Na]^+$.

(5S)-(+)-5-Carbomethoxy-3-formyl-5,6-dihydroindolo[2,3-*a*]indolizine (12). Compound **7** (428 mg, 1 mmol) was stirred in a solution of 0.3 ml conc. H_2SO_4, 1 ml water and 9 ml CH_3CN at room temperature for 72 hrs. The reaction mixture was concentrated, and the residue chromatographed over silica gel. Eluates from petroleum ether (60°C to 80°C)-chloroform mixture (75:25) afforded a solid which was recrystallised from petroleum ether (60°C to 80°C)-chloroform to afford the aldehyde **12** (129 mg; 44%). m.p. 185-186°C; $[\alpha]_D^{27.2}$ +134.41° (*c* = 0.247, $CHCl_3$). The IR, UV, MS, ^{1}H NMR and ^{13}C NMR spectra were identical to that of compound **10**.

(6S)-(-)-6-Carbomethoxy-3-hydroxy-6,7-dihydroindolo[2,3-*a*]quinolizinium hydrogen sulphate (13). Further elution of the above column with 10% methanol in chloroform gave a solid which was recrystallised from methanol to afford **13** (100 mg; 34%). m.p. 278-280°C; $[\alpha]_D^{29}$ -48° (*c* = 0.1, H_2O); IR (KBr): 3432, 1735, 1626, 745 cm^{-1}; UV (MeOH): λ_{max} (log e) 205.9 (5.07), 223.8 (5.11), 263.8 (4.85), 310.0 (5.00), 322.8 (5.06), 415.5 (4.78); The ^{1}H NMR and ^{13}C NMR were identical to that of compound **11**.

Acknowledgements

Authors are thankful to C.S.I.R New Delhi, India for financial support.

References and Notes

1. Toyota, M.; Komori, Ihara, M. *J. Org. Chem.* **2000**, *65*, 7110.
2. Cox, E. D; Cook, J. *Chem. Rev.* **1995**, *95*, 1797.
3. Chang, K. P.; Fong, D.; Bray, R. S. *Biology of Leishmania and Leishmaniasis;* Elsevier Biomedical Press: Amsterdam, The Netherlands, 1985; Vol. I.
4. Manna, R. K.; Jaisankar, P.; Giri, V. S. *Synth. Comm.* **1995**, *25*, 3027.
5. In case of phenethylamine, a 1:1 mixture of diastereoisomeric mixture of isoquinolines was

obtained
6. Medda, S.; Jaisankar, P.; Manna, R. K.; Pal, B.; Giri, V. S.; Basu, M. K. *J. Drug Target.* **2003**, *11*, 123.
7. Sinha, J.; Raay, B.; Das, N.; Medda, S.; Mahato, S. B.; Banu, M. K. *Drug Delivery* **2002**, *9*, 55.