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# A β-CARBOLINE ALKALOID FROM *HEDYOTIS CHRYSOTRICHA**

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**Key Word Index**—*Hedyotis chrysotricha*; Rubiaceae; β-carboline alkaloid; chrysotricine; inhibition of HL-60 cells; X-ray chrystallography.

**Abstract**—A new β-carboline alkaloid, named chrysotricine, was isolated from *Hedyotis chrysotricha*, which is a traditional Chinese medicine. Its structure was elucidated by spectroscopic and X-ray diffraction analysis. Chrysotricine exhibited inhibitory activity against the growth of HL-60 cell *in vitro*. © 1997 Elsevier Science Ltd

## INTRODUCTION

More than 20 species of the genus *Hedyotis* have been used in traditional Chinese medicine [1]. We have reported flavonoids from *H. lindleyana* and iridoid glucosides from *H. chrysotricha* [2–4]. In the present paper, we report the isolation and structural determination of a new β-carboline alkaloid, named chrysotricine, from *H. chrysotricha*. Chrysotricine is only a trace alkaloid (0.0001%).

## RESULTS AND DISCUSSION

Alcoholic extracts of whole plants of *H. chrysotricha* were subjected to macroreticular resin and silica gel chromatography repeatedly to yield the alkaloid, chrysotricine (**1**).

Chrysotricine (**1**) gave a positive reaction with Dragendorff's reagent and emitted a strong blue fluorescence under UV light. The UV absorptions, $\lambda_{max}$ nm 255, 309 and 376, were characteristic of β-carboline alkaloids [5]. The $M_r$ (338) from EI-mass spectrometry and elemental analyses, led to the molecular formula $C_{21}H_{26}N_2O_2$. In the down-field region of the $^1$H NMR spectrum (Table 1), four vicinal aromatic H's at $\delta$ 8.18 (1H, *dt*, $J$ = 1.2, 8.1 Hz), 7.14 (1H, *ddd*, $J$ = 1.0, 6.8, 8.1 Hz), 7.52 (1H, *ddd*, $J$ = 1.2, 6.8, 8.5 Hz) and 7.79 (1H, *dt*, $J$ = 1.2, 8.1 Hz) and two H's as an AB-system at $\delta$ 7.82 (1H, *d*, $J$ = 6.4 Hz) and 8.13 (1H, *d*, $J$ = 6.4 Hz), indicated the presence of only one substituent at C-3 of the β-carboline skeleton, alongside an *N*-methyl group. However, interpretation was hampered by large changes of some signals when $^1$H and $^{13}$C NMR spectra were measured in different solvents ($CDCl_3$ *vs.* $CD_3OD$). Finally, the structure (Fig. 1) of chrysotricine was determined by X-ray diffraction analysis; its absolute configuration was not established in the present work.

Further analysis of the $^1$H and $^{13}$C NMR of chrysotricine showed that the spectra measured in $CDCl_3$ were consistent with the crystal structure. In the spectra measured in $CD_3OD$, additional CH signals at $\delta_C$ 86.4 and $\delta_H$ 3.78 (*s*) were observed, with concomitant disappearance of the $CH_2$ signals at $\delta_C$ 39.5 and $\delta_H$ 3.18 and $\delta_H$ 3.88 observed in $CDCl_3$. This suggested that the structure of chrysotricine had been converted into **2** in methanol (Fig. 2), a typical case of tautomerism. The AB signals at $\delta_H$ 3.18 and 3.88 appeared as two broad humps, instead of the usually sharp doublets. This is probably caused by hindered rotation. According to HR-mass spectra, the fragmentation pathways of chrysotricine may be rationalized as shown in Fig. 3.

The general biosynthetic pathway of indole alkaloids involves condensation of tryptamine with secologanin to afford strictosidine and vicoside [6]. Further elaboration engenders eight types of indole alkaloids. The structure of chrysotricine suggested the union of tryptamine with a monoterpene linalyl oxide (Fig. 4) which is widely distributed in plants, such as in the genus *Citrus* and *Thea*, etc. This provided a new viable biogenesis of indole alkaloids. Thus, it is highly possible that chrysotricine has the 15*R*, 18*R*-configuration as shown in Fig. 2, which follows from one of the two naturally occurring linalyl oxides with the

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Table 1. $^1H$ and $^{13}C$ NMR data of chrysotricine in $CDCl_3$ and $CD_3OD$

| | $CDCl_3$ | | $CD_3OD$ | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 2 | 141.6 | | 143.4 | |
| 3 | 157.2 | | 157.5 | |
| 5 | 129.5 | 8.02 (*d*) | 128.2 | 7.82 (*d*, $J = 6.4$) |
| 6 | 114.4 | 8.09 (*d*) | 115.1 | 8.13 (*d*, $J = 6.4$) |
| 7 | 131.1 | | 132.4 | |
| 8 | 131.1 | | 132.4 | |
| 9 | 122.0* | 8.13 (*d*, $J = 8.1$) | 123.0 | 8.18 (*dt*, $J = 1.2, 8.1$) |
| 10 | 119.0 | 7.21 (*t*) | 118.9 | 7.14 (*ddd*, $J = 1.0, 6.8, 8.1$) |
| 11 | 129.5 | 7.59 (*t*) | 129.5 | 7.52 (*ddd*, $J = 1.2, 6.8, 8.5$) |
| 12 | 118.0 | 7.61 (*d*) | 118.8 | 7.79 (*dt*, $J = 1.0, 8.5$) |
| 13 | 146.8 | | 147.0 | |
| 14 | 39.5 | 3.18 (*br*) | 86.4 | 3.78 (*s*) |
| | | 3.88 (*br*) | | |
| 15 | 85.6 | | 86.3 | |
| 16 | 37.3 | 2.24 (*m*) | 38.3 | 2.19 (*m*) |
| | | 2.15 (*m*) | | 1.96 (*m*) |
| 17 | 26.0 | 1.74 (*m*) | 27.2 | 1.72 (*m*) |
| | | 1.49 (*m*) | | 1.47 (*m*) |
| 18 | 87.2 | 4.27 (*m*) | 88.3 | 3.20 (*m*) |
| 19 | 70.3 | | 71.7 | |
| 20 | 45.2 | 4.42 (3 H, *s*) | 45.6 | 4.44 (3H, *s*) |
| 21 | 28.7 | 1.40 (3H, *s*) | 29.1 | 1.38 (3H, *s*) |
| 22 | 27.5 | 1.12 (3H, *s*) | 26.2 | 1.12 (3H, *s*) |
| 23 | 24.0 | 1.02 (3H, *s*) | 25.4 | 1.07 (3H, *s*) |

TMS as int. standard, 500 Hz for $^1H$ and 125 MHz for $^{13}C$ NMR.

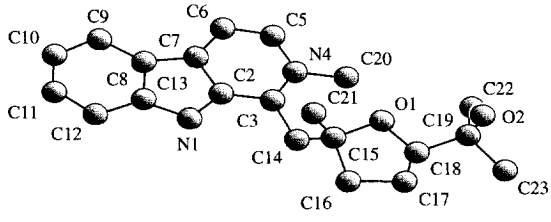


Fig 1. X-ray structure of chrysotricine.

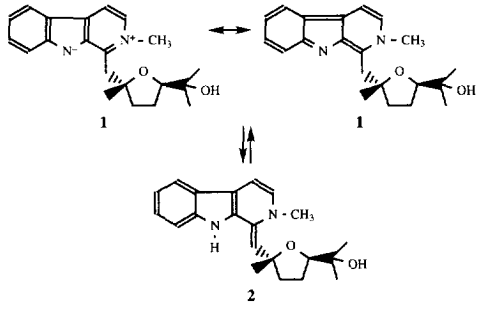


Fig 2. Structural inter-convertion of chrysotricine in $CDCl_3$ and $CD_3OD$.

methyl group and 2-hydroxy-2-propyl group in *cis*-relationship [7].

Pharmacological investigations revealed that chrysotricine inhibited the growth of HL-60 cells *in vitro*. The inhibition rate was 63% at 10 $\mu$M.

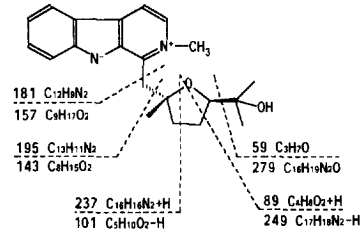


Fig 3. Fragmentation pathways of chrysotricine.

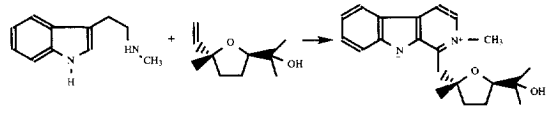


Fig 4. Putative biogenetic pathway of chrysotricine.

## EXPERIMENTAL

*General.* Mps are uncorr. UV spectra were measured in MeOH, IR spectra in KBr. $^1H$ NMR were obtained at 500 MHz and $^{13}C$ NMR at 125 MHz, with TMS as int. standard. EIMS were measured at 70 eV with a direct inlet system. Silica gel (150–200 mesh) and GF254 were used for CC and TLC, respectively.

*Plant material.* Whole plants of *H. chrysotricha* were collected from Anhui province (China) in August 1993. A voucher specimen is deposited in the Her-

barium of Wuhu College of Traditional Chinese Medicine.

*Extraction and isolation.* Cut and air-dried whole plants (38 kg) were extracted with 95% EtOH. The extract was concd and the residue dissolved in $H_2O$. After filtration, the filtrate was fractionated in a macroreticular resin column eluting with $H_2O$, 25% EtOH and 95% EtOH. The fr. eluted with 95% EtOH was dissolved in $CHCl_3$–MeOH (47:3). The deposit was removed and the soln subjected to CC on silica gel eluting with mixts of $CHCl_3$ and MeOH of sequentially increasing polarity. The fr. (2.5 g) eluted with 20% MeOH was rechromatographed over silica gel VLC eluting with EtOAc–MeOH (4:1)+2% HOAc in MeOH. Chrysotricine was obtained from the acidic MeOH eluted part.

*Chrysotricine* (**1**). Yellow prisms, mp 160–161°. $[\alpha]_D$ +26° (MeOH, *c* 0.05). Strong bright blue fluorescence under UV light. UV $\lambda_{max}$ nm: 255, 309, 376. IR $\nu$(KBr) $cm^{-1}$: 2990, 1624, 1600, 1450, 1340, 1277, 1155, 1050, 748, 762. Elemental analyses: found: C, 74.53; H, 7.70; N, 8.32. $C_{21}H_{26}N_2O_2$ requires: C, 74.56; H, 7.69; N, 8.28. EIMS *m/z* (rel. int): 338 $[M]^+$ (4), 320 (4), 279 (11), 249 (3), 237 (37), 221 (10), 196 (100), 195 (10), 181 (5), 154 (6), 143 (2), 85 (5), 71 (12). HRMS: 338.1990 ($C_{21}H_{26}N_2O_2$ $\Delta = -0.4$ mmu), 196.1003 ($C_{13}H_{12}N_2$ $\Delta = +0.3$ mmu), 181.0762 ($C_{12}H_9N_2$, $\Delta = -0.4$ mmu).

*X-ray analysis.* Chrysotricine was recrystallized from $Me_2CO$ to give transparent yellow prisms. Triclinic, space group P1, cell dimensions a = 7.782 (4), b = 8.127 (3), C = 8.441 (4) A, $\alpha = 99.89$, $\beta = 101.63$ (4), $\gamma = 116.04$ (3)°, V = 444.8 (3) $A^3$, z = 1, Dx = 1.252 $mg/m^{-3}$. Intensity data were collected on an R3m/E diffractometer with graphite monochromatized MoK$\alpha$ radiation using the $\omega$-$2\theta$ scan technique, $2\theta$ range 2–48°. 1370 independent reflections were obtained, from which 1320 were treated as observed with $F^2 \geqslant 3\sigma(F^2)$. The crystal structure was solved by direct methods using the SHELX-86 program [8]. The positions of all non-H atoms were located in the chosen E-maps, and were refined anisotropically by the full matrix least squares method, and the type of C, H and O atoms were determined. Difference Fourier syntheses and geometric calculation were used to determine the positions of H atoms. After refinement by least squares procedure, the final $R_f = 0.035$, $R_w = 0.035$.

*HL-60 cell growth inhibition assay.* HL-60 cells ($1 \times 10^5$ $ml^{-1}$) were cultured for 48 hr with chrysotricine at various concs in PRMI-1640-supplemented with 10% calf serum in a $CO_2$ incubator at 37°. After incubation, viable cell numbers were determined by the Trypan Blue dye exclusion method.

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