

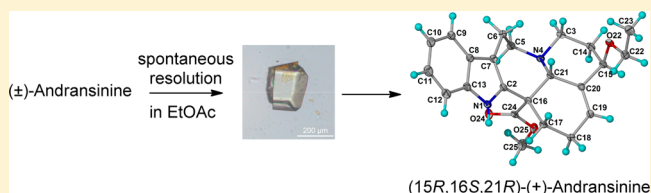
Andransinine: An Unusual Case of Spontaneous Resolution in an Indole Alkaloid Derivative

Yun-Yee Low,* Chew-Yan Gan,[†] and Toh-Seok Kam*

Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Supporting Information

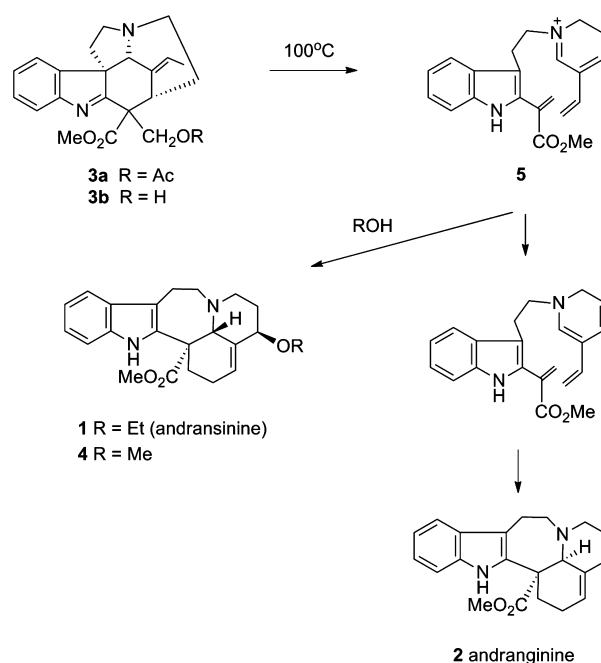
ABSTRACT: Racemic andransinine (1), an indole alkaloid derivative obtained during isolation of alkaloids from *Alstonia angustiloba* and *Kopsia pauciflora*, was found to undergo spontaneous resolution when crystallized in EtOAc, forming racemic conglomerates (an equimolar mechanical mixture of enantiomerically pure individual crystals). X-ray analyses of the enantiomers (obtained from crystals from EtOAc solution and from chiral-phase HPLC) provided the absolute configuration of each enantiomer as (15*R*,16*S*,21*R*)-(+)-andransinine (1a or I+) and (15*S*,16*R*,21*S*)-(–)-andransinine (1b or I–).



We recently reported the isolation of andransinine (1), [α]_D –8 (c 0.13, CHCl₃), from the leaf extract of *Alstonia angustiloba*.¹ We have now isolated andransinine (1) as an optically inactive racemate from *Kopsia pauciflora*. Analysis of the MS and NMR spectroscopic data led to structure 1 for andransinine,¹ which showed structural similarity to the rare indole alkaloid andranginine 2, previously isolated as an optically inactive alkaloid from *Cratogeomys verticillatus*.² The structure and relative configuration of 2 were confirmed by X-ray diffraction analysis.³ A nonenzymatic pathway, possibly involving a 4+2 cycloaddition of a secodine-type precursor, was presented to account for the isolation of the racemic alkaloid.² Such a proposal was supported by the observation that thermolysis of the putative precursor precondylocarpine acetate (3a) at 100 °C in EtOAc solution resulted in the formation of racemic andranginine (2), while carrying out the thermolysis in the presence of MeOH led to the formation of the methoxy derivative 4 (Scheme 1).² In view of the presence of an ethoxy group in 1, the use of denatured ethanol as extracting solvent (albeit under mild conditions at ambient temperature), and the presence of precondylocarpine (3b) among the alkaloids isolated, the possibility that 1 arose in a similar manner to 4, via interception of the iminium intermediate 5 (Scheme 1) during isolation of the alkaloids, cannot be discounted.

Since andransinine (1) crystallized readily from EtOAc to give good-quality colorless block crystals (mp 212–214 °C), we carried out an X-ray diffraction analysis (Mo K α radiation, crystal I), which confirmed the structure deduced from the NMR data. The crystal system is monoclinic, with a space group of P2₁ (a chiral space group).

Crystallization of 1 in a chiral space group was initially puzzling, as it suggested the possibility that 1 is an enantiomerically pure compound.^{4–7} This is in contrast to the parent alkaloid, andranginine (2), which was obtained as an optically inactive racemate. The crystal system of 2 is monoclinic, with the observed space group of P2₁/c (a centrosymmetric space group), which is also consistent with a

Scheme 1. Formation of Andranginine (2) from Precondylocarpine Acetate (3a)²

racemate.³ We surmised that the earlier detection of optical activity in the sample of 1 from *Alstonia* (which contributed to the initial confusion) was likely a result of racemate contamination, and this was confirmed upon chiral-phase HPLC analysis, which showed the presence of minor impurity peaks in addition to the two enantiomers in the sample of 1 from *Alstonia*, compared to the present sample from *Kopsia*, for

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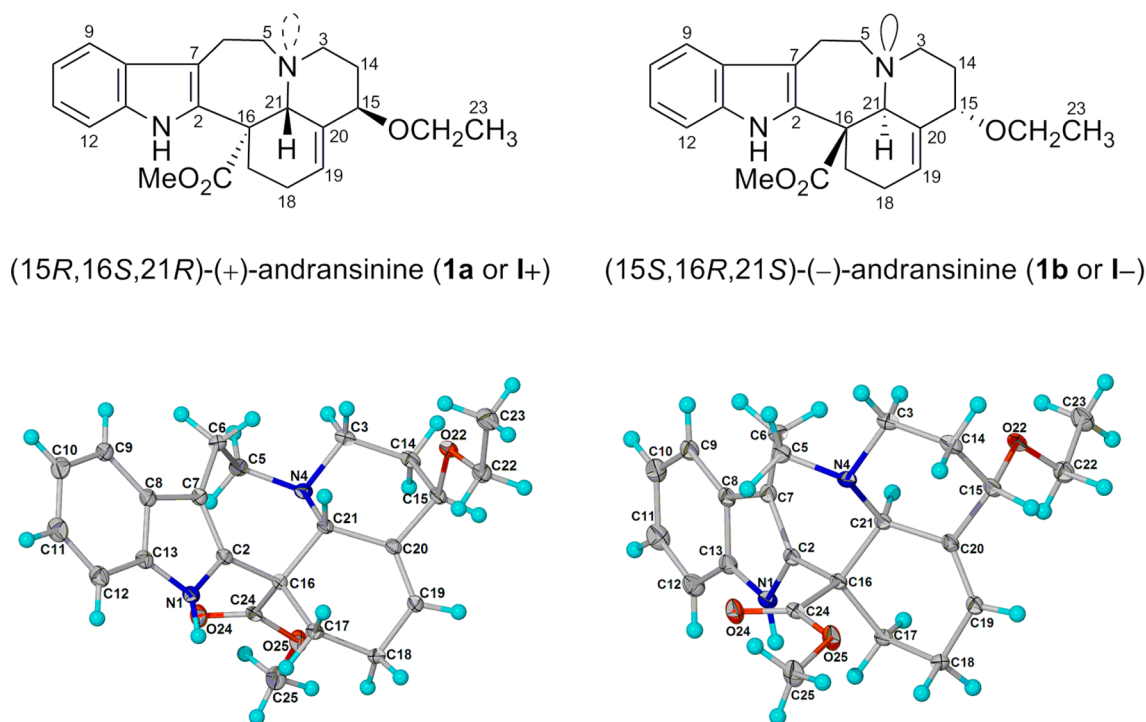


Figure 1. Left: X-ray crystal structure of (15R,16S,21R)-(+)-andransinine (**1a** or **I+**) [Flack parameter, $\alpha = -0.04(0.16)$; Hooft parameter, $y = -0.03(0.04)$]. Right: X-ray crystal structure of (15S,16R,21S)-(-)-andransinine (**1b** or **I-**) [Flack parameter, $\alpha = -0.02(0.10)$; Hooft parameter, $y = 0.04(0.06)$].

which the HPLC chromatogram showed only two peaks, corresponding to the two constituent enantiomers.

Crystallization of **1** in several other solvent systems, such as CH_2Cl_2 /hexanes and MeOH, yielded crystals morphologically different from those crystallized from EtOAc and also possessed different melting points compared to those crystallized from EtOAc. The crystals obtained from EtOAc solution had a higher melting point (**I**, 212–214 °C) when compared with those obtained from CH_2Cl_2 /hexanes (**II**, 186–190 °C) and MeOH (**III**, 204–206 °C). X-ray diffraction analyses were carried out for these crystals (**II** and **III**), which revealed that they correspond to a racemic compound or crystalline racemate.⁸

It is evident that crystals of **1** from EtOAc solution (**I**) constitute a racemic conglomerate (an equimolar mechanical mixture of crystals, each one of which contains only one of the two enantiomers present in a racemate), while crystals from CH_2Cl_2 /hexanes (**II**) or MeOH (**III**) solutions correspond to a racemic compound (or a crystalline racemate, in which the two enantiomers are present in equal quantities in a well-defined arrangement within the crystal lattice).^{4–7} Andransinine (**1**) obtained during isolation of alkaloids from *A. angustiloba* and *K. pauciflora* are therefore racemates, which spontaneously resolved to form racemic conglomerates in EtOAc, but formed racemic compound crystals in CH_2Cl_2 /hexanes or MeOH.^{4–7}

Since (\pm)-andransinine crystallized from EtOAc are racemic conglomerates, any one of the individual crystals will yield an absolute configuration when the X-ray diffraction analysis is carried out with Cu $K\alpha$ radiation.^{4–7} Accordingly, a suitably large crystal (ca. $0.43 \times 0.35 \times 0.28$ mm) was picked from an EtOAc solution containing conglomerates of **1**. The crystal was cut in half (ca. $0.20 \times 0.35 \times 0.28$ mm), and this half-crystal was subjected to an X-ray diffraction analysis using Cu $K\alpha$ radiation. The absolute configuration and structure of this

crystal were determined as (15R,16S,21R)-andransinine. The remaining half of the andransinine crystal (ca. $0.23 \times 0.35 \times 0.28$ mm) was dissolved in a minimum amount of EtOH and analyzed by chiral-phase HPLC, which showed that it corresponded to the first peak of the chromatogram (t_R ca. 4 min). The first peak in the HPLC chromatogram therefore corresponds to (15R,16S,21R)-andransinine (**1a**), while the second peak (t_R ca. 8 min) corresponds to its enantiomer, (15S,16R,21S)-andransinine (**1b**).

As attempts to obtain pure enantiomers (in sufficient amounts to determine the specific rotation) from the conglomerate by mechanical sorting under the microscope were unsuccessful due to lack of sufficient morphological differentiation, separation of both enantiomers was also carried out by chiral-phase HPLC,⁹ after which X-ray diffraction analysis (Cu $K\alpha$) was carried out on the pure enantiomers obtained, in order to confirm the absolute configuration of the slower eluting enantiomer in HPLC. The absolute configuration and the specific rotation corresponding to each enantiomer are shown in Figure 1.

In conclusion, (\pm)-andransinine (**1**), an indole alkaloid derivative obtained during isolation of alkaloids from *A. angustiloba* and *K. pauciflora*, was found to exhibit polymorphism, as well as to undergo spontaneous resolution when crystallized in EtOAc. This represents a rare, if not the first, report of such a phenomenon in alkaloids.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on a Mel-Temp melting point apparatus and were uncorrected. Optical rotations were recorded on a JASCO P-1020 digital polarimeter. HPLC was performed on a Waters liquid chromatograph with a Waters 600 controller and a Waters 2489 tunable absorbance detector. A Chiralpak AD-H column (4.6×150

mm, Daicel, Japan) packed with amylose tris(3,5-dimethylphenylcarbamate) coated on 5 μ m silica gel was used, at ambient temperature, and fractions were collected manually.

(\pm)-Andransinine (1): colorless block crystals from EtOAc; mp 212–214 °C; colorless needles from CH₂Cl₂/hexanes; mp 186–190 °C; colorless lath crystals from MeOH; mp 204–206 °C. Isolation and full characterization data of andransinine have been previously reported.¹

X-ray Crystallographic Analysis. X-ray diffraction analysis was carried out on a Bruker SMART APEX II CCD area detector system equipped with a graphite monochromator and a Mo K α fine-focus sealed tube (λ = 0.71073 Å), at 100 K, or on an Agilent Technologies SuperNova Dual CCD area detector system equipped with mirror monochromator and using Cu K α radiation (λ = 1.54184 Å), at 100 K. The structure was solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on F^2 (SHELXL-2014). All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with relative isotropic parameters. The absolute structures were determined by refinement of the Flack parameter¹⁰ and computation of the Hooft parameter.¹¹ Crystallographic data for all five structures (**I**, **II**, **III**, **I+**, **I−**) have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).

X-ray Diffraction and Chiral-Phase HPLC Analyses of a Single Crystal of Andransinine (1) Obtained from EtOAc Solution. A suitably large crystal (ca. 0.43 \times 0.35 \times 0.28 mm) was selected from the crystals obtained in EtOAc solution. It was cut in half (ca. 0.20 \times 0.35 \times 0.28 mm). X-ray diffraction analysis using Cu K α radiation was carried out on this half-crystal. The remaining half of the andransinine crystal (ca. 0.23 \times 0.35 \times 0.28 mm) was dissolved in a minimum amount of EtOH and analyzed by chiral-phase HPLC (*n*-hexane/EtOH/Et₂NH, 85:15:0.2, flow rate, 0.8 mL/min). A single peak was observed corresponding to a retention time of 3 min 47 s.

Separation of Enantiomers 1a and 1b by Chiral-Phase HPLC. (\pm)-Andransinine (**1**) (2.5 mg) was dissolved in EtOH (0.15 mL) and resolved using a chiral column (eluting solvent: *n*-hexane/EtOH/Et₂NH, 85:15:0.2; flow rate 0.8 mL/min; 30 injections, 5 μ L each) to yield two fractions. Fraction 1: retention time 3 min 51 s, 1 mg, [α]_D²⁵ +85 (c 0.1, CHCl₃). Fraction 2: retention time 7 min 52 s, 1.1 mg, [α]_D²⁵ −75 (c 0.1, CHCl₃). X-ray diffraction analyses were also carried out on the crystals (from EtOAc) of the pure enantiomers.

Crystallographic data of (\pm)-andransinine (**I**) obtained from EtOAc with Mo K α radiation: C₂₃H₂₈N₂O₃, M_r = 380.47, monoclinic, space group $P2_1$, a = 8.4914(10) Å, b = 9.1548(11) Å, c = 12.4788(15) Å, β = 95.838(3)°, Z = 2, D_{calcd} = 1.309 g cm^{−3}, crystal size 0.40 \times 0.36 \times 0.12 mm³, $F(000)$ = 408, Mo K α radiation (λ = 0.71073 Å), T = 100 K. The final R_1 value is 0.0373 (wR_2 = 0.0932) for 3997 reflections [$I > 2\sigma(I)$]. Flack parameter, x = 0.6(0.5). CCDC number: 994845.

Crystallographic data of (\pm)-andransinine (**II**) obtained from CH₂Cl₂/hexanes: C₂₃H₂₈N₂O₃, M_r = 380.47, monoclinic, space group $C2/c$, a = 39.082(4) Å, b = 8.5880(11) Å, c = 24.128(3) Å, β = 105.802(7)°, Z = 16, D_{calcd} = 1.297 g cm^{−3}, crystal size 0.54 \times 0.25 \times 0.03 mm³, $F(000)$ = 3264, Mo K α radiation (λ = 0.71073 Å), T = 100 K. The final R_1 value is 0.0573 (wR_2 = 0.1292) for 2452 reflections [$I > 2\sigma(I)$]. CCDC number: 994645.

Crystallographic data of (\pm)-andransinine (**III**) obtained from MeOH: C₂₃H₂₈N₂O₃·CH₃OH, M_r = 412.52, orthorhombic, space group $Pna2_1$, a = 8.6828(2) Å, b = 21.4082(4) Å, c = 11.2277(2) Å, Z = 4, D_{calcd} = 1.313 g cm^{−3}, crystal size 0.84 \times 0.36 \times 0.24 mm³, $F(000)$ = 888, Mo K α radiation (λ = 0.71073 Å), T = 100 K. The final R_1 value is 0.0352 (wR_2 = 0.0923) for 4579 reflections [$I > 2\sigma(I)$]. CCDC number: 994646.

Crystallographic data of (15*R*,16*S*,21*R*)-(+)-andransinine (**1a** or **I+**): C₂₃H₂₈N₂O₃, M_r = 380.47, monoclinic, space group $P2_1$, a = 8.5096(1) Å, b = 9.1538(1) Å, c = 12.5333(1) Å, β = 96.030(1)°, Z = 2, D_{calcd} = 1.301 g cm^{−3}, crystal size 0.35 \times 0.28 \times 0.20 mm³, $F(000)$ = 408, Cu K α radiation (λ = 1.54184 Å), T = 100 K. The final R_1 value is

0.0347 (wR_2 = 0.0911) for 3742 reflections [$I > 2\sigma(I)$]. Flack parameter, x = −0.04(0.16), Hooft parameter, y = −0.03(0.04). CCDC number: 994647.

Crystallographic data of (15*S*,16*R*,21*S*)-(−)-andransinine (**1b** or **I−**): C₂₃H₂₈N₂O₃, M_r = 380.47, monoclinic, space group $P2_1$, a = 8.5089(2) Å, b = 9.1505(2) Å, c = 12.5241(2) Å, β = 96.049(2)°, Z = 2, D_{calcd} = 1.303 g cm^{−3}, crystal size 0.20 \times 0.15 \times 0.10 mm³, $F(000)$ = 408, Cu K α radiation (λ = 1.54184 Å), T = 100 K. The final R_1 value is 0.0301 (wR_2 = 0.0801) for 3903 reflections [$I > 2\sigma(I)$]. Flack parameter, x = −0.02(0.10), Hooft parameter, y = 0.04(0.06). CCDC number: 994648.

■ ASSOCIATED CONTENT

Supporting Information

Chiral-phase HPLC chromatogram of (\pm)-andransinine (**1**) (obtained from *A. angustiloba* and *K. pauciflora*) and of **1a** or **I+** (half-crystal from EtOAc solution). Packing diagram for **I**. X-ray crystallographic data in CIF format for **I**, **II**, **III**, **I+**, and **I−**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Authors

* (Y. Y. Low) Tel: 603-79675165. Fax: 603-79674193. E-mail: yylow@um.edu.my.

* (T. S. Kam) Tel: 603-79674266. Fax: 603-79674193. E-mail: tskam@um.edu.my.

Present Address

[†]University Malaysia of Computer Science and Engineering, Jalan Alamanda 2, Precinct 1, 62000 Putrajaya, Federal Territories, Malaysia.

Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Professor Ward T. Robinson, University of Canterbury, Christchurch, New Zealand, on the occasion of his 77th birthday.

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