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Assignment of the absolute configuration of (−)-linarinic acid by theoretical calculation and asymmetric total synthesis

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Abstract—The specific rotation of (−)-linarinic acid calculated using Hartree-Fock and density functional theory on 14 representative conformers of this molecule were all negative. The most stable conformer was found to be almost identical to the crystallographic structure. These results are consistent with the assumption that (−)-linarinic acid has a (1*S*)-configuration. An asymmetric total synthesis with starting products chosen on the basis of the theoretical calculations was found to corroborate the theoretical assignment. On the basis of the theoretical calculations of specific rotations and a total asymmetric synthesis, the absolute configuration of the natural product (−)-linarinic acid was determined to be 1*S*.

1. Introduction

Linaria vulgaris Mill. (Scrophulariaceae), distributed in the northeast and Inner Mongolia of China, is known as a folk medicine for antitussive expectorant, antiasthmatic, diuretic, laxative and curing headache, jaundice and dermatosis.[1] (−)-Linarinic acid, [1,2,3,9-tetrahydro-pyrrolo(2,1-*b*)quinazolin-1-carboxylic acid] is a new compound isolated from the ethanol extract of the whole herb of *L. vulgaris* Mill. by Hua et al.[2]

Using UV, IR, NMR, MS, 2D NMR, and X-ray data analysis, the structure was identified as a new tricyclic quinazoline alkaloid.[2] A notable characteristic of the compound is the existence of a 1-carboxylic and functional group, which results in a stereogenic carbon. A single-crystal X-ray diffraction analysis of this compound was performed and its result deposited with the Cambridge Structural Database.[3,4] However, the absolute configuration of the stereogenic carbon could not be assigned. We decided to combine recent advances in the calculation of specific rotations with a total asymmetric synthesis in order to determine the absolute configuration of (−)-linarinic acid.

According to the biosynthetic regular pattern of alkaloids, (−)-linarinic acid was biosynthesized from a certain amino acid. It is well known that almost all naturally existing amino acids are of the (*S*)-configuration. Thus, it has been assumed that (−)-linarinic acid also has an (*S*)-configuration. Herein, we calculated the specific rotation of (*S*)-linarinic acid using a recently developed theoretical approach implemented in Dalton 2.0 for accurate calculations of specific rotations.[5] Based on the agreement of the calculated sign of the specific rotation with the experimentally observed specific rotation of the natural product, an asymmetric total synthesis of (*S*)-linarinic acid was carried out using *o*-nitrobenzaldehyde and L-glutamic acid as the starting material. The absolute configuration of (−)-linarinic acid was unambiguously assigned as having an (*S*)-configuration by both theoretical calculations and asymmetric total synthesis.

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2. Results and discussion

2.1. Theoretical calculation

The first ab initio calculations of specific rotations were reported in 1997 by Polavarapu.[6] Since then, a number of investigations have been published.[7,8] Recently, theoretical calculations of specific rotations were used successfully to assist synthetic chemists in elucidating the absolute configuration of some chiral molecules.[9] The theoretical calculations proved particularly successful for molecules with no more than two stereogenic centers when combined with other theories such as van't Hoff's rule, which is applicable to rather complex natural products.[9] Here, we use theoretical calculations to assign the absolute configuration for (−)-linarinic acid.

(−)-Linarinic acid has only one stereogenic center. There is therefore no need to apply van't Hoff's rule and a direct calculation of its specific rotations will suffice to determine the absolute configuration of the molecule. In addition to being strongly basis set dependent, the specific rotation is also known to depend strongly on the molecular geometry and in particular on the molecular conformation.[10] Based on a conformational search and cluster analysis, 14 stable and representative conformations were identified (Chart 1).

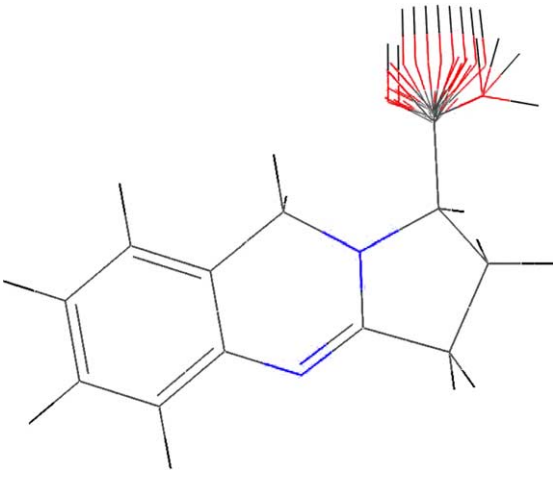


Chart 1. 14 Representative conformers of (*S*)-linarinic acid.

These conformations were optimized using the Hartree-Fock method with a 6-31G** basis set. The specific rotations of these conformations were calculated using several basis sets, of which the largest was the aug-cc-pVDZ set, in accordance with the recommendations of Stephens et al.[11] The specific rotations of the optimized conformers were evaluated using the Dalton quantum chemistry package[12] with Hartree-Fock and density functional theory with the B3LYP hybrid functional.[11] The results obtained at the Hartree-Fock and DFT levels of theory using the aug-cc-pVDZ basis are collected in Table 1. In Chart 2, we have illustrated the specific rotation obtained for the different basis sets at the Hartree-Fock level of theory.

Table 1. Theoretical specific rotations at the sodium D-line of 14 representative conformers of (*S*)-linarinic acid using the aug-cc-pVDZ basis set

Conformer	HF	DFT/B3LYP
1	−58.98	
2	−57.64	
3	−40.12	
4	−71.41	−119.88
5	−271.08	−491.94
6	−243.34	−435.72
7	−213.87	−355.86
8	−142.90	−269.63
9	−115.38	−189.02
10	−88.78	−124.10
11	−79.35	−83.25
12	−75.14	−73.48
13	−115.15	−313.05
14	−83.34	−250.13

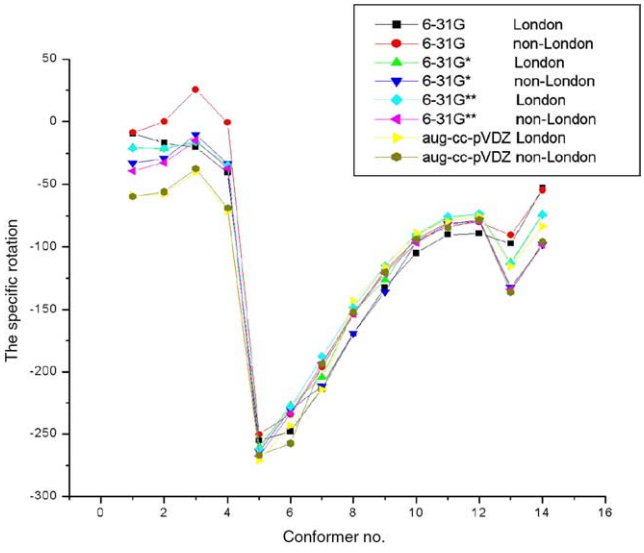


Chart 2. Specific rotations of (*S*)-linarinic acid calculated at the HF level with different basis sets.

As shown in Table 1, all conformers give rise to a negative specific rotation at both the Hartree-Fock and DFT/B3LYP levels of theory (the only exceptions to this observation were conformers 1 and 3 using the (inadequate) 6-31G basis set at the Hartree-Fock level of theory). This sign is in agreement with the experimental observations for (−)-linarinic acid. London atomic orbitals[13] have been used in our calculation to ensure that the $G_{\alpha\beta}$ tensor determining the optical rotation is independent of the gauge origin.[14–16]

The crystal structure of (−)-linarinic acid is available in the Cambridge Structural Database with deposition number CCDC 242576. This crystal structure is almost identical to the most stable conformer given by the conformational search with an rmsd of 0.603 (Chart 3). A Hartree-Fock calculation of the specific rotation was also carried out for this structure, giving a specific rotation of −263. This indicates that the conformational search gave a correct and reasonable set of

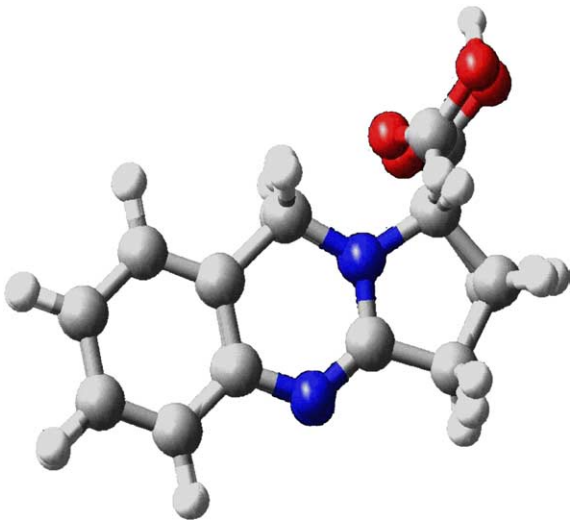


Chart 3. The alignment of crystal structure and most stable structure given by conformation search.

conformations, and that the most stable conformer found was indeed responsible for the largest contribution to the overall specific rotation.

Carboxylic acids are known to dimerize in low concentration; and this may affect our calculated optical rotations, as shown by Polavarapu.[17] However, considering the good agreement obtained between theory and experiment for (−)-linarinic acid, we do not believe this to be a serious concern for this molecule.

Considering that predictions of rotation at a single wavelength do not reveal the full story, we also calculated the specific rotations at different wavelengths and compared them with the experimental data. This is shown in Table 2. This comparison again confirmed that our calculations are correct.

In summary, the theoretical calculations of most conformers at the HF and DFT levels gave a negative sign for the optical rotation, which is consistent with the natural product. These results strongly suggest that the absolute configuration at carbon-1 for natural (−)-linarinic acid is *S*.

Table 2. Comparison of the theoretical specific rotations at four different wavelengths of 14 representative conformers of (*S*)-linarinic acid using the aug-cc-pVDZ basis set with experimental data

Wavelength	589	578	546	436
Experimental	−290	−300	−340	−750
Calculations	−356	−375	−443	−993

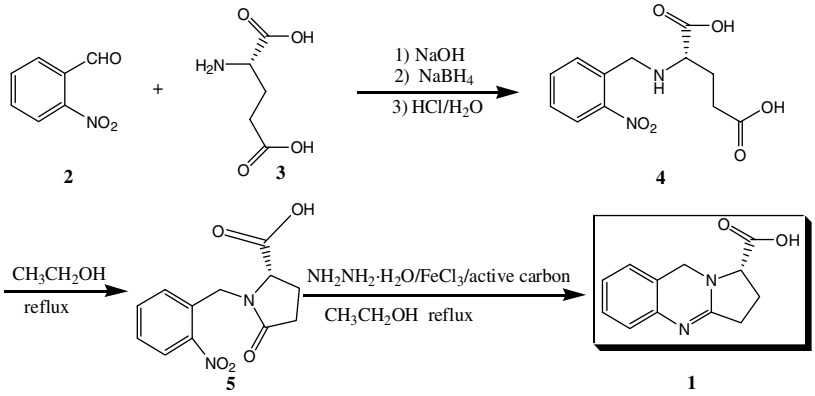
2.2. Chemical synthesis

Different synthetic routes were attempted to synthesize the target compound. In order to synthesize the stereoisomers asymmetrically and on the basis of the theoretical calculations described in the preceding section, L-glutamic acid was used as the chiral block and *o*-nitrobenzaldehyde as the starting material.

First, *o*-nitrobenzaldehyde and L-glutamic acid were transformed into compound **4** via a reductive aminated reaction in 65.9% yield.[18] Compound **4** was then refluxed in ethanol for 3 h and dehydration gave compound **5** in 97.1% yield.[18] The two above-mentioned products were used in the following steps without further purification. Reductive approaches using catalytic hydrogenation[19] and $SnCl_2/HCl$[20] failed due to poor solubility in most solvents. However hydrazine hydrate, ferric chloride, and active carbon were used to reduce the nitro group. The product obtained was confirmed to be our target compound (*S*)-linarinic acid in 59.6% yield (Scheme 1).

In this synthetic scheme, (*S*)-linarinic acid was obtained in an unexpected way. The mechanism of the last step is still unknown and further investigations of this mechanism are in progress.

The specific rotations of the intermediates and (*S*)-linarinic acid were measured. The minus sign of the specific rotation of (*S*)-linarinic acid $\{[\alpha]_D^{18} = -290.0$ (*c* 0.01, MeOH)} was consistent with that of the natural product $\{[\alpha]_D^{18} = -217.0$ (*c* 0.01, MeOH)}.[2] Based on the above comparison, we concluded that the absolute configuration at the C-1 position of natural (−)-linarinic acid is an (*S*)-configuration (Chart 4).



Scheme 1. Synthetic route to (−)-linarinic acid.

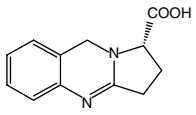


Chart 4. The structure of (*S*)-linarinic acid.

3. Conclusions

Using both theoretical calculations and an asymmetric total synthesis of (*S*)-linarinic acid, the determination of the absolute configuration for natural (−)-linarinic acid has been accomplished herein. The specific rotation of (*S*)-linarinic acid calculated by ab initio methods were all negative. Based on this result, the absolute configuration of natural (−)-linarinic acid was predicted to be (1*S*). On the basis of theoretical calculations, the asymmetric synthesis of (*S*)-(−)-linarinic acid was achieved in three steps in an overall yield of 38.1%. A comparison performed between the natural product and the synthesized product demonstrated that the sign of the specific rotation of the synthesized product was consistent with the natural product. We therefore concluded that the absolute configuration at C-1 in naturally existing (−)-linarinic acid is (1*S*).

4. Experimental

4.1. Conformational search and calculations of optical rotations

The conformational search of (*S*)-linarinic acid was performed using Systematic Search by MMFFs force field in SYBYL[21] by rotating two bonds of the carboxyl group. Water was used as the 'solvent' in the conformational search. A cluster analysis was then accomplished based on those conformations to give 14 representative conformers.

These 14 representative conformers obtained from the cluster analysis were optimized at the HF/6-31G** level using the Dalton program, and the corresponding specific rotations were calculated at the frequency of the sodium D-line (589.3 nm), also using Dalton[12] at the HF and DFT levels of theory, respectively. The specific optical rotation is calculated as follows:[5]

$$[\alpha]_D = 0.1343 \times 10^{-3} \beta \bar{\nu}^2 / M$$

with β in units of bohr.[4] M is the molar mass in g/mol, and $\bar{\nu}$ is the frequency of the incident light in cm^{-1}. β is the electric dipole-magnetic dipole polarizability, calculated by

$$\beta = \frac{1}{3\omega} \sum_{\alpha} (G'_{\alpha\alpha})$$

where ω is the frequency of the incident light, and G' is the response function

$$G'_{\alpha\beta} = -2 \sum_{n \neq 0} \frac{\omega}{\omega_n^2 - \omega^2} \mathrm{Im}[\langle \psi_0 | \mu_\alpha | \psi_n \rangle \langle \psi_n | m_\beta | \psi_0 \rangle]$$

where ω_n is the excitation energy from the electronic ground state ψ_0 to the excited state ψ_n, and μ_α and m_β are components of the electric and magnetic dipole operator, respectively.

Four basis sets were used for the calculation of the specific rotation: 6-31G, 6-31G*, 6-31G**, and aug-cc-pVDZ. The final specific rotation was determined by Boltzmann weighting of specific rotations for all adopted conformers.

4.2. Organic general

All melting points were determined on a Büchi-540 melting point apparatus and are uncorrected. The IR spectra were recorded on a Bruker IR S-55 instrument. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AC (E)-300 instrument using tetramethylsilane as an internal standard. EI-MS was obtained on Finnigan LCQ–MS spectrometer. ESI-MS was obtained on Agilent 1100 series LC/MS Trap. Specific rotation was measured on Perkin–Elmer 241MC Polarimeter. Analytical thin-layer chromatography (TLC) was performed on precoated plates of silica gel HF254 (0.5 mm, Qingdao, China). Flash column chromatography was performed on silica gel H (60 μm, Qingdao, China).

4.2.1. Preparation of (*S*)-(−)-2-(2-nitro-benzylamino)-pentanedioic acid 4. L-Glutamic acid (**3**) (9.80 g, 66.7 mmol) was added at room temperature to a solution of sodium hydroxide (2 M, 50 mL). Then an ethanol solution (150 mL) of *o*-nitrobenzaldehyde **2** (8.30 g, 54.9 mmol) was added dropwise. The mixture was stirred at room temperature for 30 min and cooled to 0 °C. Sodium borohydride (1.20 g, 32.4 mmol) was added in small portions at 0–5 °C. The mixture was stirred for 90 min at room temperature, and another portion of *o*-nitrobenzaldehyde (1.70 g, 11.2 mmol) added. After 20 min, a second portion of sodium borohydride (0.31 g, 8.1 mmol) was added as before, and the mixture stirred for 45 min at room temperature. The resulting solution was extracted with ether (150 mL × 3). The aqueous layer was acidified to pH 2–3 at 0–5 °C with concentrated hydrochloric acid. The resulting suspension was filtered to give a white dusty solid 12.40 g, yield 65.9%. Mp 186.1–190.0 °C. $[\alpha]_D^{18} = +47.7$ (*c* 0.51, DMSO). IR ν_{max} (KBr) cm^{-1}, 3422, 3040, 1708, 1601, 1530, 1438, 1343, 1260, 1178, 860, 813, 792, 740. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.76 (2H, m), 2.30 (2H, m), 3.07 (1H, t, $J = 5.4$ Hz), 3.85 (1H, d, $J = 14.8$ Hz), 4.07 (1H, d, $J = 14.9$ Hz), 7.52 (1H, m), 7.70 (2H, m), 7.93 (1H, d, $J = 9.8$ Hz), 9.25 (2H, s). EI-MS *m*/*z*: 264 (0.79), 237 (11.48), 218 (14.39), 136 (100.00), 130 (24.68), 119 (10.15), 92 (12.42), 78 (84.02), 65 (27.40), 51 (23.85), 39 (29.31).

4.2.2. Preparation of (*S*)-(+)-1-(2-nitro-benzyl)-5-oxo-pyrrolidine-2-carboxylic acid 5. The suspension of 2-(2-nitro-benzylamino)-pentanedioic acid **4** (12.01 g, 42.5 mmol) in ethanol (420 mL) was heated at reflux for 3 h. The resulting solution was filtered and concentrated in vacuum to give an ivory-white dusty solid 10.91 g, yield 97.1%. Mp 193.9–196.6. $[\alpha]_D^{18} = +10.2$ (*c*

0.50, DMSO). IR ν_{max} (KBr) cm^{-1}, 3411, 2805, 2515, 1743, 1645, 1578, 1521, 1457, 1407, 1335, 1260, 1217, 975, 791, 728, 677. ^1H NMR (DMSO-d_6, 300 MHz) δ 2.03 (1H, m), 2.41 (2H, m), 4.08 (1H, d, $J = 8.9$ Hz), 4.38 (1H, d, $J = 16.8$ Hz), 5.01 (1H, d, $J = 16.8$ Hz), 7.43 (1H, d, $J = 7.7$ Hz), 7.55 (1H, t, $J = 7.6$, 7.8 Hz), 7.72 (1H, t, $J = 7.5$, 7.6 Hz), 8.04 (1H, d, $J = 8.1$ Hz), 13.00 (1H, s). ESI-MS m/z: 265.1 (M^++1).

4.2.3. Preparation of (*S*)-(−)-linarinic acid 1. To a solution of 1-(2-nitro-benzyl)-5-oxo-pyrrolidine-2-carboxylic acid **5** (10.00 g, 37.6 mmol) in ethanol (360 mL) was added active carbon (1.00 g) and a catalytic amount of ferric chloride (0.95 g, 3.5 mmol). Then the suspension was heated to 50 °C and hydrazine hydrate (5.52 mL, 113.5 mmol) was added dropwise. The mixture was heated at reflux for about 64 h. Then the mixture was filtered and the solvent was removed by vacuum evaporation. The residue was purified by flash chromatography on silica gel using methanol–ethyl acetate (5:4, v/v) to give a white solid 4.87 g, yield 59.6%; mp 229.9–232.5 °C dec. $[\alpha]_D^{18} = -290.0$ (c 0.01, MeOH). IR ν_{max} (KBr) cm^{-1} 3420, 2925, 1666, 1602, 1586, 1500, 1376, 1292, 771. ^1H NMR (CD_3OD, 300 MHz) δ 2.22–2.31 (1H, m), 2.54–2.62 (1H, m), 2.98–3.10 (2H, m), 4.30 (1H, dd, $J = 9.3$, 4.1 Hz), 4.75 (1H, d, $J = 15.4$ Hz), 4.96 (1H, d, $J = 16.3$ Hz), 7.00 (1H, m), 7.17–7.25 (2H, m), 7.28–7.33 (1H, m). ^{13}C NMR (CD_3OD, 75 MHz) δ 25.56, 30.22, 46.46, 70.17, 117.77, 118.43, 128.09, 128.29, 130.28, 132.34, 165.00, 174.92. ESI-MS m/z: 217.0 (M^++1), 215.0 (M^+−1). EI-MS m/z: 216 (59.47), 215 (62.09), 171 (41.22), 169 (46.73), 145 (11.58), 144 (100.00), 129 (7.91), 118 (26.71), 91 (9.34), 77 (19.57), 51 (17.81), 39 (17.30).

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