**Solid-state characterization of optically pure (+)Dihydromycetin extracted from
Ampelopsis grossedentata leaves**

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Abstract

Dihydromyricetin (DMY) is a natural flavanol compound isolated from a traditional Chinese medicine, *Ampelopsis grossedentata*. Despite that optically pure (+)DMY is desired for treating chronic pharyngitis and alcohol use disorders, only DMY racemate is commercially available due to prolonged exposure time to high temperature and the presence of metal ions during industrial extraction, which cause racemization of the homochiral (+)DMY. We have developed an extraction method for successfully obtain optically pure (+)DMY. We have further assessed the physicochemical properties of the two phases using PXRD, DSC, TGA, FTIR, and moisture sorption. Among them, PXRD and FT-IR are suitable for quickly distinguishing homochiral (+)DMY from racemic ($\pm$)DMY. Lastly, with the aid of cocrystallization with theophylline, the absolute configuration of homochiral (+)DMY was identified to be (2R, 3R).

Keywords: extraction, enantiomer, chirality, solid-state characterization, dihydromyricetin molecular configuration

Introduction

The single enantiomers of drugs are preferred in drug development, because they are more efficacious and with fewer side effects.^{1,2} For examples, L-carnitine and S-levodopa showed desired therapeutic effects, but D-carnitine had high toxicity and R-levodopa associated with granulocytopenia.^{3,4} L-propranolol, S-ofloxacin, and R-warfarin showed remarkably higher activities than their opposite enantiomers^{5,6,7} and the natural S-folinic acid showed five-fold higher bioavailability than the other enantiomer.⁸ For drugs that are marketed as a racemate, developing and launching single enantiomer based products is a strategy that provides an additional commercial opportunity for both innovative and generic companies.⁹ Consequently, chiral separation and comprehensive characterization of optically pure enantiomers of active drug substances are highly desired. Optically pure enantiomers may be obtained by asymmetric syntheses, diastereomeric salt/cocrystal formation, chiral chromatography, preferential crystallization, or enzymatic separation.¹⁰

Plant-derived flavonoids exhibit a variety of biological activities and thereby are appealing candidates for drug discovery and nutraceutical development. Flavonoids are chiral like many other bioactive molecules extracted from plants. However, only three marketed flavanol compounds are claimed as resolved enantiomers, i.e., S(-) eriodictyol, S(-) homoeriodictyol, and (2R, 3R) taxifolin.¹¹ Among them, only (2R, 3R) taxifolin has been marketed as a drug in Russia, and the commercial S(-) eriodictyol has been shown to be actually a racemate.¹²

Dihydromyricetin (DMY) is the main flavanol compound isolated from a traditional Chinese medicine, *Ampelopsis grossedentata*, which has a reported content of DMY as high as 39.4% (w/w).¹³ Previous studies suggested a strong intrinsic activity and efficacy of DMY as a therapeutic component for various diseases, including alcohol use disorders and chronic pharyngitis.¹⁴ It also showed beneficial pharmacological activities, including hepatoprotective, antioxidative, anti-inflammatory and antihypertensive effects.¹⁵ Currently, DMY capsules are sold in US as a nutraceutical supplement to prevent hangovers. A recently completed clinical study showed that DMY can improve glucose and lipid metabolism in patients with non-alcoholic fatty liver disease.¹⁶

DMY has two chiral centers and thus has four potential enantiomers (Figure 1). Not surprisingly, homochiral (+)DMY showed higher efficiency than (±)DMY in increasing the level of the cytokine interleukin-10 in lipopolysaccharide treated mice, indicating higher anti-inflammatory activity.¹⁷ Moreover, pharmacokinetics studies demonstrated that homochiral (+)DMY is eliminated from the body more slowly, and a slightly higher AUC_{0-∞} is observed.¹⁸ However, despite the intense effort for the commercial development of this compound, only two crystal structures of DMY were reported, (±)DMY

dihydrate and hexaacetate.^{19,20} Both are racemic compound between (2R, 3R) and (2S, 3S) enantiomers. It should be noted that the ($\pm$)DMY dihydrate can also be named a tetrahydrate of the racemic di-compound, ($\pm$)DMY, because the asymmetric unit contains (2R, 3R) DMY, (2S, 3S) DMY, and water in 1:1:4 ratio.¹⁹

Among the available methods, extraction from the plants containing naturally synthesized optically pure flavonoid compounds is a convenient approach to produce the pharmacologically relevant pure enantiomer. However, commercial DMY products primarily comprise ($\pm$)DMY due to racemization during the extraction process. We found that the chiral inversion of DMY is accelerated by the presence of metal, high pH, and high temperature.²¹ As such, there is a need for a process that would allow successful extraction of homochiral (+)DMY from *Ampelopsis grossedentata* leaves. In addition, the absolute configuration of the homochiral (+)DMY is not yet known. Correspondingly, the purpose of this work was threefold: 1) develop an improved extraction process for (+)DMY from *Ampelopsis grossedentata*; 2) characterize homochiral (+)DMY and racemic ($\pm$)DMY to guide the development of analytical methods for distinguishing them in solid-state; 3) determine the absolute configuration of (+)DMY.

Figure 1. Molecular structure of DMY and four possible enantiomers

Materials

Seven commercial DMY samples (purity > 98%) were purchased from Sigma-Aldrich (USA), Aladdin Inc. (Shanghai, China), Dekang Biochem (Zhejiang, China), Microherb Inc. (Hunan, China), Kangbaotai Fine Chemicals Inc. (Hubei, China), Huakang Biotechnology Development Inc. (Hunan,

China), Miaoyao Biotechnology Inc. (Guizhou, China). Homochiral DMY (purity > 99%) was also isolated from the *Ampelopsis grossedentata* in the laboratory using a procedure developed by us (see Methods section).

Methods

Extraction procedure

200 g *Ampelopsis grossedentata* powders (Chongqing Youxiantea Inc. Chongqing, China) was suspended in 1.5 L of 75% ethanol (v/v) aqueous solution and sonicated with ultrasound for 30 min at 60°C. The solution was then filtered through 30-50 µm filter paper (Whatman 102, Hangzhouwohua, Zhejiang, China) placed in a Buchner funnel. The filtrate was dried by rotary evaporation at 50°C. The dried solid dissolved in 1.0 L hot deionized water with 0.1% activated carbon and then filtered through 30-50 µm filter paper while still hot. The filtered aqueous solution was cooled to precipitate crystals. The cooling crystallization was repeated five times, and the sample was dried in an oven at 50 °C. The identity and purity of this compound were determined by ¹H-NMR and chiral HPLC. Theophylline anhydrous was received from BASF (Ludwigshafen, Germany). Racemic (±)DMY monohydrate was prepared by suspending the racemic dihydrate in ethanol, which was stirred with a magnetic stirring bar under ambient conditions for 24 h. The resulting solid was then recovered by vacuum filtration and dried at room temperature.²² All solvents were HPLC grade. All compounds were used as received without further purification.

Chiral HPLC Analysis

The chromatographic system consisted of a Waters e2698 (Waters Corp, Milford, USA) and a DAD detector (2998 photodiode array detector, Waters Corporation, USA). Separation was achieved using a Apollo C₁₈ column (250 mm × 4.6 mm, 5 µm, Alltech Associates, Deerfield, IL, USA) using a mobile phase consisting of menthol/water/phosphoric acid/β-cyclodextrins (30:68.1:0.1:1.8) at a flow rate of 1 mL/min. DAD detection was performed at 290 nm for DMY. Specificity and sensitivity of this method were previously established, and the peak resolution was better than 1.5.²¹

(+) DMY-theophylline cocrystallization

A mixture of 1:1 stoichiometric amount of (+)DMY (0.2 mmol) and theophylline (0.2 mmol) was suspended in 5 mL of acetonitrile in a covered glass beaker and stirred with a magnetic stirring bar under

ambient conditions for 24 h. The suspension was vacuum filtered through a 0.45 μm membrane. The filtrate was allowed to slowly evaporate undisturbed through a small hole in parafilm covering the glass vial at room temperature. Single crystals suitable for X-ray structure elucidation were obtained after 1 week.

Single-Crystal X-ray Data Collection and Structure Determination

Single crystal X-ray diffraction (SCXD) was carried out on a Bruker diffractometer at 173 K using Cu-K α radiation (graphite monochromator). Data analysis was performed using a suite of software from Bruker, including APEX, SADABS, and SAINT.²³ The structures were solved by direct methods and refined on F^2 by full-matrix least-squares methods using the SHELXTL program package.²⁴ All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were located either from the difference Fourier map or at calculated positions and allowed to ride on their parent atoms in the refinement cycles.

Room Temperature Powder X-ray Diffractometry

Powders were analyzed on a Siemens 5005 powder diffractometer with Cu K α radiation (1.54056 Å), pre-calibrated using a silicon standard. Samples were scanned between 2θ from 5 to 35°, with a step size of 0.02° at 1 s/ step. The tube voltage and current were 45 kV and 40 mA, respectively.

Variable Temperature Powder X-ray Diffractometry

A variable temperature stage (TTK 450; Anton Paar, Graz-Straßgang, Austria) was used to control the sample temperature on a powder X-ray diffractometer (D8 ADVANCE; Bruker AXS, Madison, WI) equipped with Cu K α radiation (40 kV $\times$ 40 mA) and Si strip one-dimensional detector (LynxEye; Bruker AXS, Madison, WI). The sample was heated to the target temperature at a rate of 10 °C/min and then maintained under isothermal conditions during data collection (5–30° 2θ with a step size of 0.02° and a dwell time of 0.5 s). The sample chamber was purged with dry nitrogen (50 mL/min) during the experiment.

Differential Scanning Calorimetry (DSC)

Samples (3~5 mg) were analyzed on a DSC Q2000 (TA Instruments), equipped with a refrigerated cooling system, under a continuous dry nitrogen purge at 50 mL/min. Data were collected from 0 °C to decomposition temperature at a heating rate of 10 °C/min. Tzero hermetically sealed aluminum pans were used for all samples. The instrument was calibrated for temperature and enthalpy change using indium.

Thermal gravimetric analysis (TGA)

Samples (~3 mg) were loaded in an open aluminum pan and heated from room temperature to 300 at 10 °C/min under dry nitrogen purge (50 mL/min) using a thermogravimetry analyzer (Model Q50, TA Instruments, New Castle, DE, USA).

Fourier transformation infrared spectroscopy (FT-IR)

FT-IR spectra of the powder samples were collected using a high resolution FT-IR spectrometer (VERTEX 70, Bruker Optics Inc., Billerica, MA, USA). Each reported spectrum is an average of 64 scans in the region of 4000-600 cm^{-1} with a resolution of 4 cm^{-1} . Data were processed by OPUS software (v5.5, Bruker Optics Inc., Billerica, MA, USA).

Dynamic Water Vapor Sorption Isotherm (DVS)

Water sorption-desorption isotherms were obtained using an automated moisture balance (Intrinsic DVS, Surface Measurement Systems Ltd., Allentown, PA, USA) at 25 °C. The nitrogen flow rate was 50 mL/min. During a routine analysis, a sample was equilibrated at each step with the equilibration criteria of either $\text{dm}/\text{dt} \leq 0.003\%$ or maximum equilibration time of 6h. However, both dm/dt and the maximum equilibration time were adjusted as needed. Once one of the criteria was met, the relative humidity (RH) was changed to the next target value following a 0% - 95% - 0% RH cycle with a step size of 5% RH. Prior to the experiment, the sample was dried *in situ* by purging with N_2 until a constant weight was obtained.

Results and discussion

Extraction of (+)DMY

The development of a successful extraction process should consider both purity and yield. Our preliminary results showed that 1) DMY is highly soluble in ethanol (~170 mg/mL at 25°C) and hot water (~20 mg/mL at 80°C) but insoluble in cold water (0.32 mg/mL at 25°C); 2) DMY degraded rapidly in hot water and alkaline solution;²⁵ and 3) high temperature and metal concentration accelerated the racemization of homochiral DMY.²¹ We had shown that in a solvent containing Fe^{3+} (2.5 mmol/L) at 60°C, the enantiomeric excess (e.e.) will decrease from 99.5% to 38.3% within one hour.²¹ Therefore, though high temperature favors the yield of extraction process, it also decreases the purity, and thus the

approach of achieving a yield by using a high temperature is counter-productive. As such, we have developed an extraction procedure to obtain substantially optically pure (+)DMY (Figure 2).

Figure 2. Extraction process of homochiral and racemic DMY from leaves of *Ampelopsis grossedentata*

Chiral purity of commercial samples

A typical chiral HPLC profile of commercial DMY products and the DMY prepared in-house is shown in Figure 3. Five of the seven commercial DMY samples have equal moles of the two enantiomers, i.e., (+)DMY and (-)DMY (Table 1), because both the optical rotation by polarimetry and ΔE by circular dichroism of those commercial DMY samples were zero (Figure S1b). The two other commercial samples contain a substantial amount of (-)DMY. In contrast, the homochiral (+)DMY prepared in this study had a single peak, corresponding to the second peak of racemic ($\pm$)DMY (Figure 3). In addition, the polarimetry result was $+32.46^\circ$, and ΔE of circular dichroism showed a negative peak at 292 nm and positive peaks at 210, 226, 260, 329 nm (Figure S1c). Thus, the commercial samples were mostly ($\pm$) DMY, but our sample was optically pure (+) DMY.

Figure 3. Typical chiral HPLC analysis profiles of DMY, a) (±)DMY and b) (+)DMY

Table 1. Enantiomeric excess (e.e.) of (+)DMY in various DMY samples.

Source	Code	Batch number	e.e. (%)
Sigma-Aldrich	A	102M4725V	0
Aladdin Inc.	B	36805	0
Dekang Biochem	C	/	0
Microherb Inc.	D	/	0
Kangbaotai fine chemicals Inc.	E	/	0
Huakang Biotechnology Development Inc.	F	/	43.4
Miaoyao Biotechnology Inc.	G	/	43.3
This work	H	/	99.5

Table 1 shows that samples **A-E** (coded, same as follow) were racemates, and samples **F** and **G** were partially racemic. In contrast, our samples were enantiomerically pure (+)DMY. The very different enantiomeric purities of these samples extracted from the same source, leaves of *Ampelopsis grossedentata*, may be attributed to several key differences in the extraction conditions (Figure 2).²⁰ 1) We used metal-free 75% ethanol in deionized water, but the other reported samples were extracted using purified water without controlling the metal concentration; and 2) during the first extraction step we used an extraction medium containing a higher ethanol concentration (75%, v/v) and at a lower temperature (60 °C) for a shorter duration (30 min). In addition to the higher enantiomer purity, our extraction

procedure also has a much higher yield of (+)DMY (~10%) than the reported process (< 2% racemic (±)DMY). Clearly, our extraction procedure is more efficient and better controlled than the prevailing commercial process.

Since non-optimized extraction processes can produce racemic DMY, it is important to develop reliable analytical methods for monitoring and quantifying the purity of DMY samples to improve the quality of commercial products by ensuring enantiomer purity. This naturally requires a reliable technique for rapid differentiation between (±)DMY and (+)DMY. Although chiral HPLC is an established technique for this purpose, it is not suitable for analyzing samples in solid state, which is considerably more convenient. Thus, we investigated the solid-state properties of racemic and homochiral DMY using the common characterization techniques of PXRD, DSC, TGA, FT-IR, and DVS.

Solid state characterization

DSC and TGA results of racemic (±)DMY dihydrate and homochiral (+)DMY are illustrated in Figures 4a and 4b. The melting points of **anhydrous** (±)DMY and (+)DMY, after dehydration, were 252.61 °C ($\Delta H = 145.4 \text{ J/g}$) and 241.27 °C ($\Delta H = 135.2 \text{ J/g}$), respectively. The wide range of melting points reported in the literature, from 240 to 256 °C,²⁶ may be attributed to the different optical and solid-state purities. The higher melting point of the racemic DMY indicates its crystal structure is more rigid. This is consistent with the slower dissolution and lower thermodynamic solubility of racemic DMY compared to homochiral DMY.²⁷ The onset dehydration event from racemic (±)DMY dihydrate is ~89.40 °C, which is completed in two steps. The total weight loss of racemic DMY dihydrate was 9.75% at 150 °C (Figure 4b), which was close to the theoretical water content of 10.01%. The DSC thermogram of homochiral (+)DMY hydrate sample shows two well separated peaks with onset temperatures of ~70.4 °C ($\Delta H = 161.5 \text{ J/g}$) and 123.7 °C ($\Delta H = 48.29 \text{ J/g}$) (Figure 4a), corresponding to two steps of weight loss in TGA, 3.61% and 5.11%, (Figure 4b). **The peak of the second endotherm in DSC thermogram occurred at about 140 °C, which is ~16 °C higher than the end of the second weight loss observed in TGA. The discrepancy is attributed to the different pan configurations, open pan and sealed pan during TGA and DSC experiments, respectively. The elevated water vapor pressure in the DSC pan after the first dehydration step stabilized the monohydrate, which led to a higher second step dehydration temperature.** The total weight loss of 8.72% at 160 °C corresponds to a 1.7 hydrate. Although we had carefully handled the sample to avoid unintended dehydration, it could have been a dihydrate, which partially dehydrated before the TGA experiments. In the ensuing discussion, we treat it as a dihydrate. The small exothermal

peak of homochiral DMY at 174.95 °C ($\Delta H = -21.33$ J/g) indicates a possible polymorphic phase change, clarification of which requires a separate investigation that was beyond the scope of this work.

Figure 4. Thermograms of racemic (±)DMY dihydrate and homochiral (+)DMY dihydrate sample, a) DSC; b) TGA

PXRD patterns of the racemic (±)DMY dihydrate, (±)DMY monohydrate, and homochiral (+)DMY sample are shown in Figure 5. Unique diffraction peaks are located at 6.74, 8.24 and 9.60° for (+)DMY powder, 9.98, 15.08 and 25.74° for (±)DMY monohydrate powder, and 5.74, 13.92 and 14.66°

for ($\pm$)DMY dihydrate powder. Based on the patterns, five commercial samples of ($\pm$)DMY from **A-E** are racemic DMY dihydrate; DMY samples **F** and **G** were physical mixtures of ($\pm$)DMY **monohydrate** and (+)DMY hydrate (Figure S2). These results are in excellent agreement with those from chiral HPLC results (Table 1). Thus, PXRD is a non-destructive and material-sparing method for quickly distinguishing homochiral (+)DMY from racemic ($\pm$)DMY.

Figure 5. Powder X-ray diffraction patterns of ($\pm$)DMY dihydrate, ($\pm$)DMY monohydrate (+)DMY dihydrate

The phase behavior of racemic ($\pm$)DMY dihydrate and homochiral (+)DMY dihydrate during heating were also further characterized using VT-PXRD (Figure 6). It can be seen that the ($\pm$)DMY dihydrate is physically stable below 75 °C (Figure 6a), which is consistent with the TGA results (Figure 5b). Some new diffraction peaks, presumably due to the ($\pm$)DMY **monohydrate**, appeared when heated to 90 °C while some peaks of the dihydrate were still visible but with much lower intensity, suggesting partial conversion of the dihydrate to the monohydrate (Figure 6a). Characteristic diffraction peaks of the dihydrate eventually disappeared at 150 °C, indicating complete conversion. Characteristic peaks of the anhydrate can also be observed at 120 °C, which indicates that dehydration of ($\pm$)DMY monohydrate to the anhydrate had already commenced before the dihydrate completely converted to the monohydrate. Thus, all three crystal forms of ($\pm$)DMY (dihydrate, monohydrate, and anhydrate) coexisted at 120 °C in

this experiment. For (+)DMY dihydrate, dehydration occurred at 60 °C and proceeded to successively form the (+)DMY monohydrate at 90 °C and (+)DMY anhydrate at 150 °C (Figure 6b). To avoid their unintended dehydration, the drying temperature should be kept at < 75°C for the (±) DMY dihydrate and <60°C for (+)DMY dihydrate.

Figure 6. VT-XRD results of a) racemic (±)DMY dihydrate and b) homochiral (+)DMY dihydrate. Unique peaks are labelled: (±)DMY monohydrate ($\neq$), (±)DMY anhydrate (*); (+)DMY monohydrate (#); (+)DMY anhydrate (Δ).

The development of successful control strategies for robust processing, handling, packaging, and storage of hydrates requires measurement of the moisture sorption and desorption behavior.²⁸ The anhydrous ($\pm$)DMY gained 7.33% of water at 10% RH, signaling monohydrate formation. The weight gain increased with RH to 10.57% at 90% RH and 12.56% at 95% RH, which is close to the theoretical value of dihydrate (11.25%). However, during the desorption process, ($\pm$)DMY dihydrate gradually losses water to a level close to that of the monohydrate at 0% RH. It lost the rest of water at 0% RH over a period of ten days. The (+)DMY anhydrate gained 0.64% of surface water at 30% RH and 8.70% at 70% RH, corresponding to the 1.7 stoichiometry observed by TGA (Figure 4b). Thus, the (+)DMY anhydrate appears to be more stable than ($\pm$)DMY anhydrate, since solid-state hydration reaction occurs at a much higher RH. During the desorption process, the (+)DMY hydrate gradually losses surface water down to 5% RH, and then the lattice water is lost when exposed to 0% RH (Figure 7). This dehydration behavior is quite different from the (+)DMY dihydrate transformation during heating, where the dihydrate losses water successively to form a monohydrate and the anhydrate (Figure 4).

Figure 7. DVS results of racemic ($\pm$)DMY dihydrate and homochiral (+)DMY dihydrate, the broken lines representing theoretical water contents of racemic dihydrate and monohydrate.

FT-IR was also used to discriminate the ($\pm$)DMY dihydrate and (+)DMY dihydrate. Characteristic peaks of (+)DMY dihydrate are at 3477.47, 3340.53, 810.06 and 950.86 cm^{-1} and ($\pm$)DMY

dihydrate are 3627.90, 3575.83, 3280.74 cm^{-1} (Figure 8). Although the spectrum of ($\pm$)DMY hydrate in the 3000-3500 cm^{-1} region is dominated by the absorption peaks of $-\text{OH}$, unique peaks are still clearly visible to identify ($\pm$)DMY hydrate. In the fingerprint region of 1300 - 600 cm^{-1} , all peaks of ($\pm$)DMY hydrate are also observed in (+)DMY hydrates with minor shift. Thus only (+)DMY can be unambiguously identified based on spectral information in this region.

In summary, the racemic ($\pm$)DMY and homochiral (+)DMY dihydrate crystals show distinct physicochemical properties. Their different stability with respect to temperature and humidity demand different downstream drying conditions to maintain solid state phase purity. Solid-state characterization techniques, i.e., PXRD, DSC, TGA, FTIR, and moisture sorption, reveal distinct differences between the two phases. In our experience, PXRD and FTIR are more suitable for monitoring phase purity of DMY samples prepared by extraction.

Figure 8. FT-IR results of racemic ($\pm$)DMY dihydrate and homochiral (+)DMY dihydrate

Determination of absolute configuration of (+)DMY

Two common methods may be used to determine the chiral configuration of homochiral (+)DMY, 1) single crystal structure determination of one of the crystal forms of homochiral (+)DMY; 2) comparing circular dichroism data to a similar chiral compound with known configurations. The first approach is preferred because it provides direct structural information. However, our efforts in growing single crystals of homochiral (+)DMY through cooling or slow evaporation from water, ethanol, methanol,

acetonitrile, and their mixtures were unsuccessful. Alternatively, we attempted cocrystallization with an achiral compound, theophylline, to access the molecular configuration of (+)DMY. The DMY is a promising cocrystal former because it has several hydrogen bonding donors and acceptors (Figure 1). In fact, we have previously discovered and characterized two cocrystals of ($\pm$)DMY,²⁹ but this is the first single crystal structure of a (+)DMY cocrystal.

The crystallographic parameters of (+) DMY-theophylline acetonitrile cocrystal, **1**, along with those of ($\pm$)DMY dihydrate are shown in Table 2. The asymmetric unit of **1** consists of theophylline, (2R,3R) DMY, and acetonitrile in 1:1:1 ratio (Figure 9a). Theophylline interacts with (+)DMY through O-H $\cdots$ N and O-H $\cdots$ O hydrogen bonds to form channels in the crystal structure to accommodate guest acetonitrile molecules (Figure 9b). The adjacent channels interact through O-H $\cdots$ O interactions between pyrogallol-pyrogallol (Figure 9c) and resorcinol-resorcinol rings (Figure 9d) to form a three-dimensional molecular host network (Figure 9e). The guest molecule, acetonitrile, interacts with the host network through C-H $\cdots$ N weak hydrogen bonding interactions. Hydrogen bonding interactions are summarized in Table S1. Both C2 and C3 of DMY were determined to have R configuration. To be certain that the configuration observed in the cocrystal is indeed that of the (+)DMY, chiral HPLC analysis was performed, which confirmed that the starting (+)DMY remains stable during cocrystallization, since the retention time corresponded to that of (+)DMY (Figure 3).

Table 2. Crystal data for ($\pm$) DMY dihydrate and theophylline-(+)DMY acetonitrile solvate

	($\pm$) DMY dihydrate (ref. 19)	(+) DMY-theophylline- acetonitrile solvate (1)
T (K)	113	173(2)
Formula	C ₁₅ H ₁₆ O ₁₀	C ₂₄ H ₂₃ N ₅ O ₁₀
Formula weight	356.28	541.47
Crystal system	Monoclinic	Orthorhombic
Space group	P-21/C	P2(1)2(1)2(1)
a (Å)	15.391(3)	6.7490(4)
b (Å)	7.9002(16)	15.2192(9)
c (Å)	24.161(5)	23.4342(14)
α (°)	90.00	90.00
β (°)	92.57(3)	90.00
γ (°)	90.00	90.00
V, Å ³	2934.8(10)	2407.0(2)
ρ , g/cm ³	1.613	1.494
Z	8	4
CCDC	667406	1452259

Figure 9. Theophylline-DMY acetonitrile solvate structure, a) asymmetric unit (chiral centers are labeled with *), b) hydrogen bond pattern that stabilizes the channel structure, with acetonitrile in the middle; c) hydrogen bonds between pyrogallol-pyrogallol and d) hydrogen bonds between resorcinol-resorcinol; e) three-dimensional molecular host network.

Conclusion

Extraction of (+)DMY from *Ampelopsis grossedentata* leaves often results in the racemization of DMY, as shown by its presence in all the commercial samples. By considering solubility, chemical stability, and racemization rate in solution, we have developed a high yield process capable of extracting pure (+)DMY, **which is a dihydrate**. We have further shown that both powder X-ray diffraction and FT-IR are suitable methods for rapid identification of homochiral (+)DMY from racemic (±)DMY. Cocrystallization of the homochiral (+)DMY with theophylline led to successful determination of the absolute configuration of two chiral centers of (+)DMY as (2R,3R).

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Supporting information

Circular dichroism results and PXRD patterns of seven commercial DMY samples; H-bond tables for theophylline-(+)-DMY acetonitrile solvate, **1**. Crystallographic data of **1** have been deposited at CSD (access code 1452259). These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Graphical Abstract