Same Formula, Different Story:

Unambiguous Identification of Stereoisomers Using Molecular Rotational Resonance Spectroscopy

Overview

For analytical chemists tasked with authentication, quality control, or structural confirmation, structural misassignment can ripple through supplier documentation, regulatory submissions, and downstream formulation decisions. As scrutiny of natural product supply chains continues to increase, so does the need for analytical methods that move beyond chromatographic retention behavior and molecular formula toward unambiguous, structure-specific confirmation. In this application note, we show how molecular rotational resonance (MRR) spectroscopy, supported by molecular modeling, enables definitive discrimination between viridiflorol, globulol, and related stereoisomers in complex mixtures, without reliance on physical reference standards.

Why MRR, Why Now

MRR spectroscopy takes a different path than traditional separation-based techniques–it probes gas-phase rotational spectra, signals that are directly tied to a molecule's three-dimensional structure. From proposed structures, BrightHub generates simulated spectra that serve as a reference library, against which experimental data can be matched to confirm identity without the need for physical reference standards.

Where GC–MS provides molecular formula, and NMR is often reserved for downstream structural confirmation, MRR bridges this gap by encoding three-dimensional structure directly into the measured spectrum. In practice, this shifts structure-level confirmation upstream, reducing dependence on downstream NMR analysis for closely related isomers. The workflow is intentionally simple **(Figure 1)**.

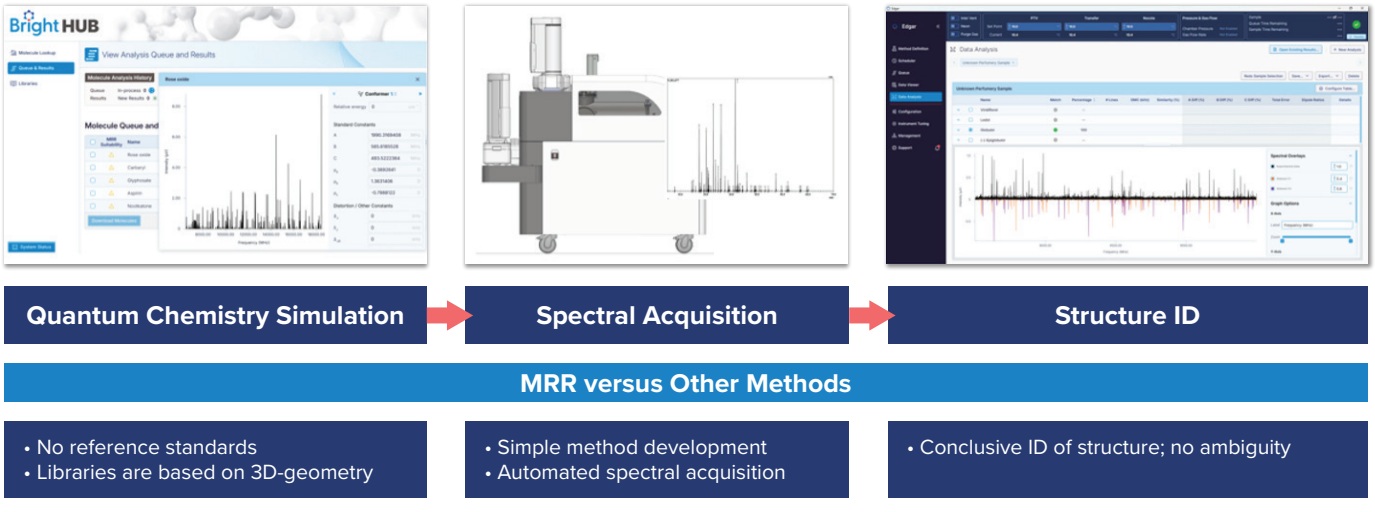

Figure 1. The spectraMRR workflow directly measures the rotational spectra of structures from complex mixtures. Unlike conventional analytical techniques that rely on chemical modification, chromatographic separation, or indirect inference from fragmentation patterns, MRR measures gas-phase rotational spectra that arise directly from a molecule's three-dimensional mass distribution. MRR provides unambiguous structure identification without derivatization, separation, or extensive method development.

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Samples can be introduced directly, and automated acquisition and spectral fitting are performed once a method is established. By focusing on a targeted set of structurally relevant components rather than comprehensive profiling, MRR enables quick, targeted screening while maintaining confidence at the structure level, making it well suited for authenticity testing, chemotype verification, and quality control applications where definitive stereochemical discrimination is essential.

Compounds like viridiflorol and globulol share the same molecular formula and a closely related tricyclic scaffold, yet differ only in stereochemistry — differences that can influence aroma profiles, biological interpretation, and claims of botanical origin. Confidently assigning structures within this class of sesquiterpene alcohols, however, remains a challenge in essential oil and natural product analysis.

In routine workflows, these compounds are identified using gas chromatography (GC)-based methods paired with mass spectral libraries. Although effective for determining molecular formula and broad classification, these approaches can fall short when asked to resolve closely related diastereomers, particularly in complex mixtures or when reference standards are unavailable or impractical.

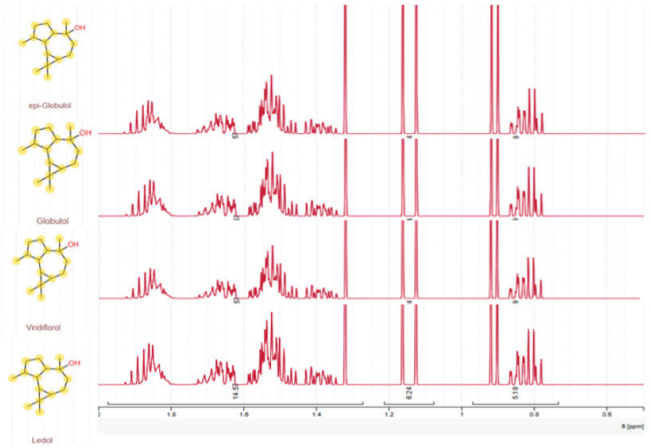

Figure 2. The 1D ^{1}H NMR spectra illustrate the inherent challenges of structure elucidation for stereoisomeric natural products using proton NMR alone. Viridiflorol, globulol, epi-globulol, and (+)-ledol are oxygenated sesquiterpenes that share an identical carbon framework and highly similar functional group composition. Structural differences among these compounds arise primarily from relative stereochemistry and hydroxyl group orientation, rather than changes in molecular connectivity. Consequently, their 1D ^{1}H NMR spectra exhibit highly overlapping chemical shift distributions, similar multiplicities, and comparable coupling patterns, reflecting closely related local magnetic environments. Although subtle differences in chemical shifts and scalar couplings are present, these variations are often insufficient for unambiguous identification based solely on 1D proton NMR. Reliable differentiation typically requires a suite of multidimensional NMR experiments (e.g., COSY, HSQC, HMBC, NOESY/ROESY), which in turn demand greater sample purity, increased acquisition time, and more extensive data analysis.

Defining Candidate Structures Prior to Spectral Analysis

Before collecting any experimental data, candidate molecular structures must be defined and simulated for spectral comparison. **Figure 3** shows how BrightHub streamlines this process by making it easy to identify and compare MRR-suitable molecules, even when multiple stereoisomers share the same molecular formula and core scaffold. Closely related stereoisomers—such as viridiflorol, globulol, ledol, and epiglobulol—are presented side by side, allowing users to quickly compare structural variants and submit more than one hypothesis for quantum-chemical calculation. The simulated rotational spectra generated from these structures form the reference library used in MRR analysis, ensuring that structure confirmation is based on a clearly defined and reproducible set of candidates rather than retrospective interpretation.

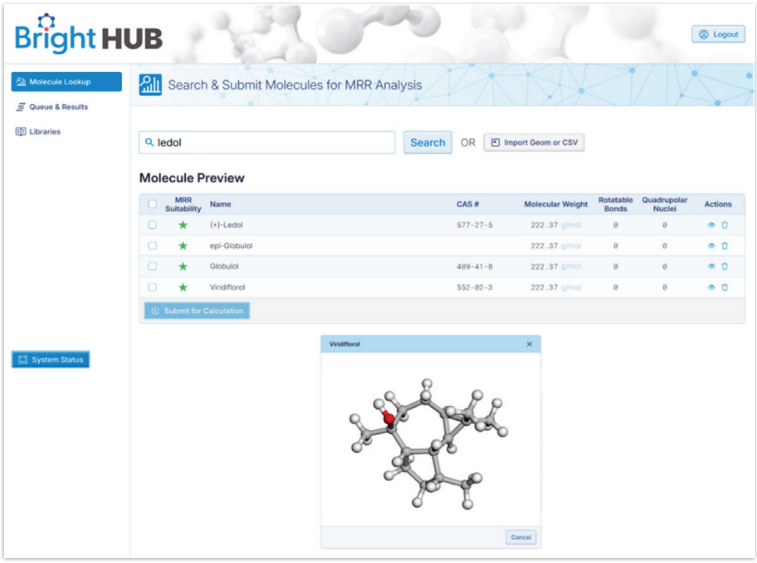

Figure 3. Candidate structure selection and submission for MRR analysis in BrightHub. The BrightHub molecular search interface displays multiple stereoisomeric candidates: (+)-ledol, epi-globulol, globulol, and viridiflorol with identical molecular weights (222.37 g/mol) and high MRR suitability. Each entry includes key metadata (CAS number, molecular weight, rotatable bonds, quadrupolar nuclei) to support feasibility assessment. Selected structures can be previewed in three dimensions and submitted directly for quantum-chemical calculations, generating simulated rotational spectra for inclusion in a theoretical reference library used in MRR-based structure identification.

Distinct Rotational Fingerprints from Subtle Stereochemical Differences

To directly test whether stereochemical differences translate into measurable spectral signatures, we examined the predicted rotational spectra of viridiflorol, globulol, and related isomers. As shown in **Figure 4**, MRR cleanly resolves what is often a persistent challenge in natural product analysis. On the left, simulated rotational spectra for each compound display distinct, non-overlapping spectral fingerprints across the same frequency window, even though the molecules share identical formulas and closely related tricyclic scaffolds. On the right, the corresponding molecular structures highlight the subtle differences in three-dimensional orientation at specific chiral centers that give rise to these spectral distinctions. Taken together, these results show that even minor stereochemical variations are sufficient for MRR to produce unique rotational signatures, enabling confident, structure-specific identification. This capability is particularly valuable for essential oil and natural product analysis, where closely related sesquiterpene alcohols often co-occur and where reliable discernment is critical for authentication, chemotype verification, and quality control—without relying on chromatographic separation or physical reference standards.

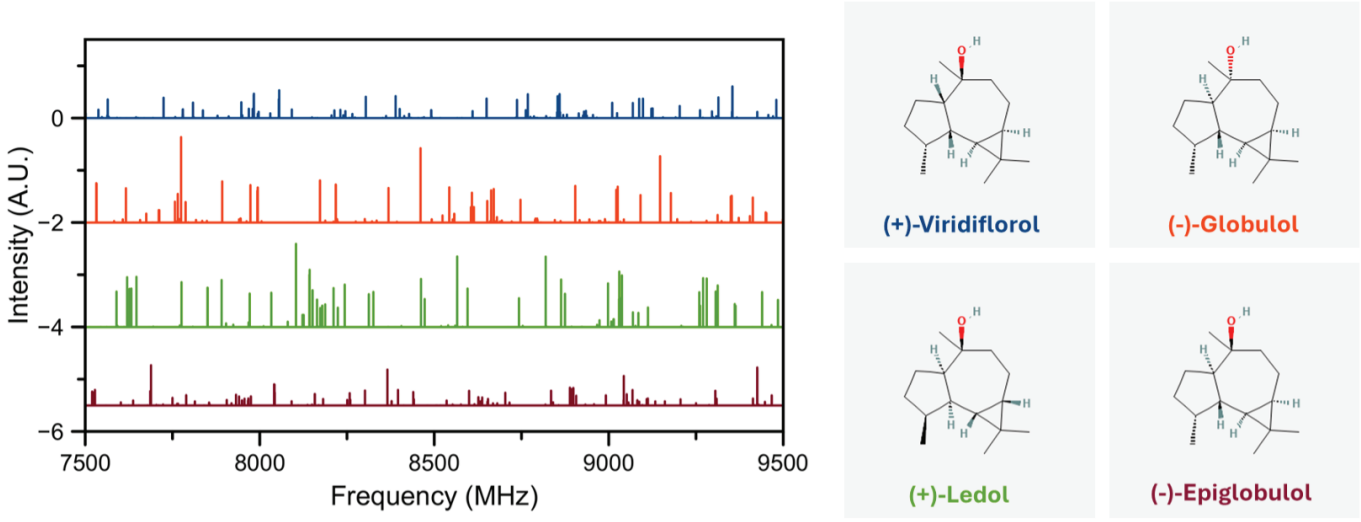

Figure 4. Distinguishing diastereomeric sesquiterpene alcohols by rotational spectroscopy. Simulated rotational spectra (left) for (+)-viridiflorol (blue), (–)-globulol (orange), (+)-ledol (green), and (–)-epiglobulol (maroon) across the 7.5–9.5 GHz frequency range show unique spectral patterns for each compound despite identical molecular formulas and shared tricyclic aromadendrane scaffolds. Corresponding molecular structures (right) illustrate the stereochemical differences among these epimers. The distinct rotational fingerprints provide the basis for definitive structure confirmation of targeted components in complex mixtures, without the need for physical reference standards.

Experimental Validation of Molecular Identity

With candidate structures defined and their rotational fingerprints established, the final step is experimental confirmation against real sample data. **Figure 5** shows how Edgar software carries out this comparison by comparing the experimentally acquired MRR spectrum of an unknown sample to the simulated spectra generated for each candidate structure. For each candidate, Edgar determines whether any matching spectral patterns are present within the expected tolerance of the theoretical modeling process.

Although several stereoisomeric possibilities are considered, the analysis converges on a single result. Globulol is identified as the sole match, showing full spectral agreement across the measured frequency range. In the spectral overlays, the experimental transitions align closely with the simulated globulol features, whereas the remaining candidates do not meet the matching criteria. This clear separation underscores the structure-specific nature of the analysis.

Together, these results represent the final confirmation stage of the MRR workflow, in which theoretical predictions are tested against experimental measurements and translated into a definitive structure assignment. For applications in which stereochemical accuracy is critical, this step provides the confidence needed to move from candidate hypotheses to a single, defensible identification.

Figure 5. Spectral fitting and structure confirmation of an unknown sample using Edgar. Edgar's data analysis interface compares experimentally acquired MRR spectra from an unknown sample against a library of simulated rotational spectra for candidate structures, including viridiflorol, ledol, globulol, and epiglobulol. The results indicate a 100% match to globulol, while other stereoisomers show no valid match. Spectral overlays (bottom) illustrate the alignment between experimental data (black) and simulated globulol transitions (colored), confirming molecular identity based on unique rotational fingerprints rather than molecular formula alone.

Conclusion

Sesquiterpene alcohols such as viridiflorol and globulol highlight a persistent challenge in natural product analysis: when molecular formula and connectivity are identical, stereochemistry becomes the defining, and often unresolved, variable. MRR spectroscopy addresses this gap by directly linking three-dimensional molecular structure to unique, measurable spectral signatures.

By pairing simulated rotational spectra with MRR measurements, the spectraMRR enables confident differentiation and confirmation of closely related epimers in complex mixtures, without chromatographic separation or physical reference standards. For analytical applications where stereochemical accuracy underpins authenticity, chemotype assignment, and quality control, the spectraMRR is a practical, structure-specific solution.

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