NMR-based identification of specialized metabolites

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Analysis of organic compound mixtures

- Steadily increasing interest for sources of renewable raw materials from **plants**
- Medicinal drugs from **plants**
- Cosmetic ingredients from **plants**
- **But**: Plants rarely produce organic compounds in chemically pure state.

- **Mixture analysis plays a central role in plant chemistry.**

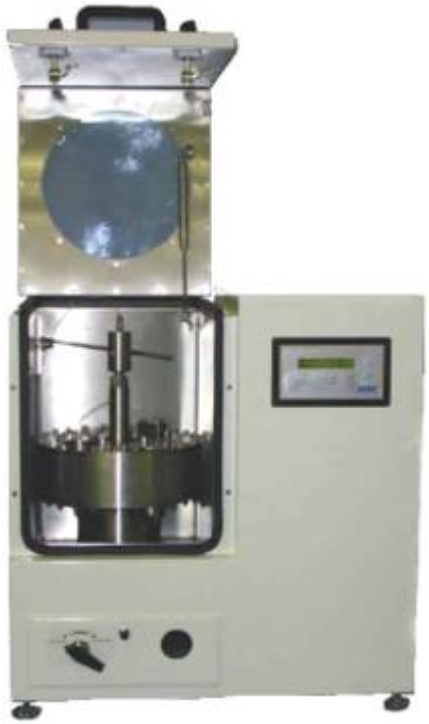
Quick identification of
known compounds
=
Structural dereplication
=
More time devoted to the
study of **new** compounds

« CARAMEL » WORKFLOW

- Extract fractionation by Centrifugal Partition Chromatography (CPC) → 10 to 15 fractions
- ^{13}C RMN spectra of fractions
- “Binning” of NMR spectra (1200 bins, each 0.2 ppm wide)
- Intensity Table: each row is related to the chemical shift value at the center of a bin and each column is related to a chromatographic fraction. Empty rows are removed from the Table.
- Row permutations in order to group chemical shift values for which chromatographic profiles are similar
- Determination of chemical shift clusters that can be assigned to a single compound
- **Compound identification by database querying**

CPC

- **C**entrifugal **P**artition **C**hromatography
- Partition of analytes between two liquid phases
- The column contains a high number of connected partition cells
- The stationary phase is maintained inside the column by action of the centrifugal force
- Analytes are injected in the first partition cells
- The mobile phase percolates through the stationary phase
- No irreversible adsorption on a solid stationary phase
- Isocratic or graduated elution, “pH-zone refining”, ion exchange
- All what goes in finally comes out, from one side or the other
- High flow rates, 20 mL/min
- Injection of 5g in a 200 mL inner volume column



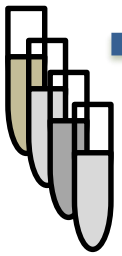
- **CPC is a preparative method**

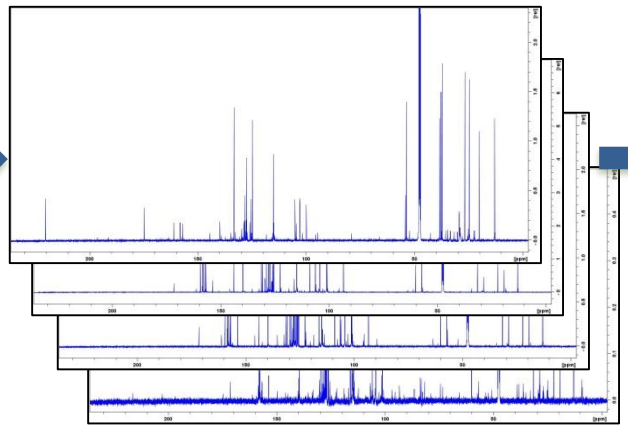
^{13}C NMR

- Fractions from CPC are analyzed by ^{13}C NMR
- One carbon atom, one peak (Unless symmetry or accident occurs)
- Low probability of peak superimposition
- Low sensitivity, presumably
- 600 MHz, cryoprobe, cooled ^{13}C coil
- ^{1}H NMR
  - Not always enough information there
  - Complex spectra due to homonuclear couplings
- Other choices:
  - Pure shift ^{1}H 1D NMR (difficult…)
  - HSQC
  - HMBC

CREATION OF THE NMR DATA TABLE

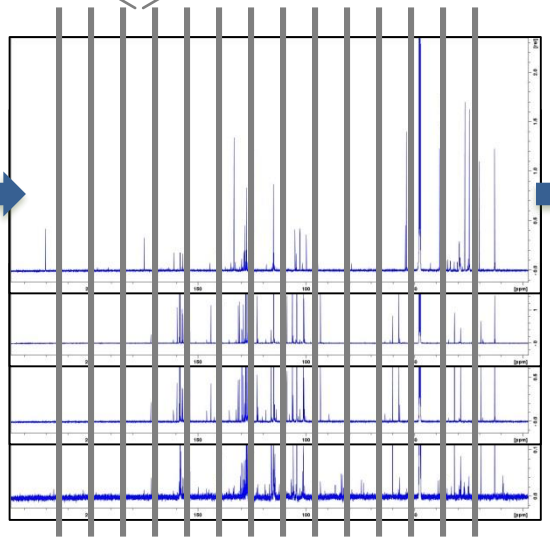
Fractions





Analysis by ^{13}C NMR

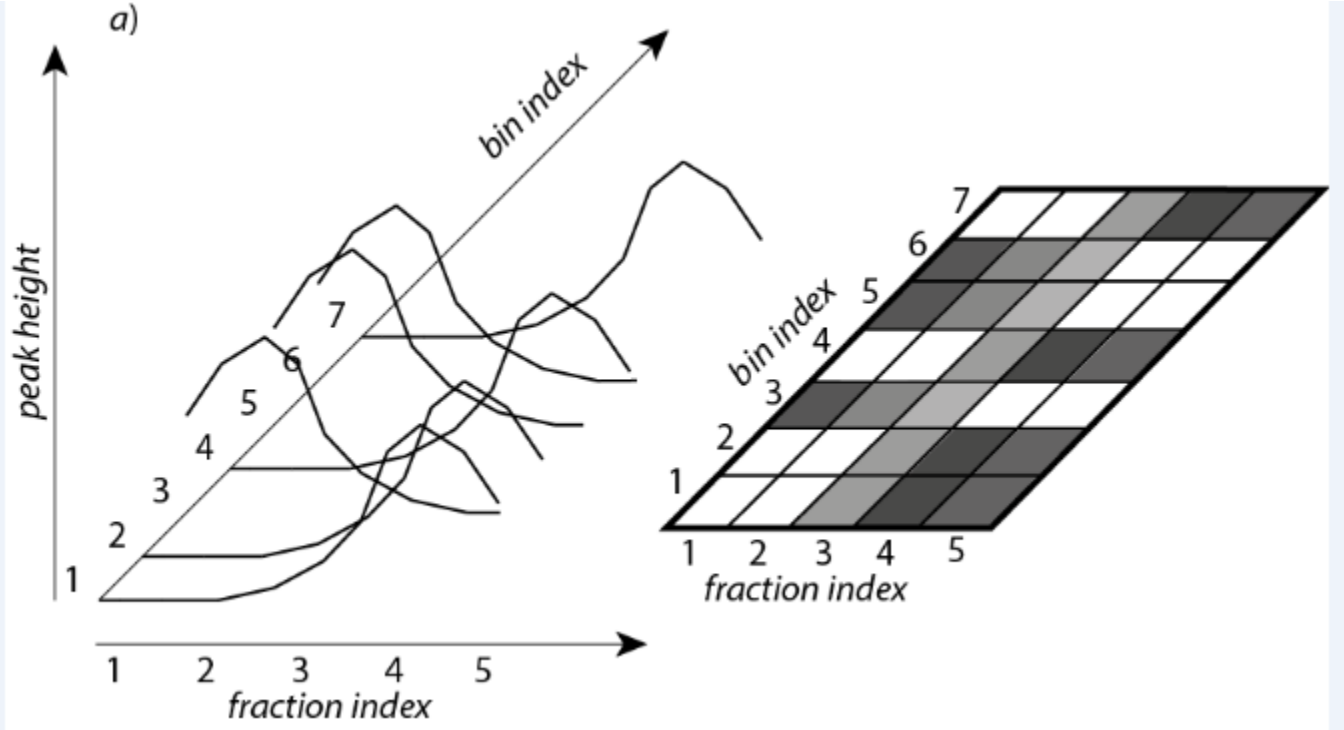
0.2 ppm



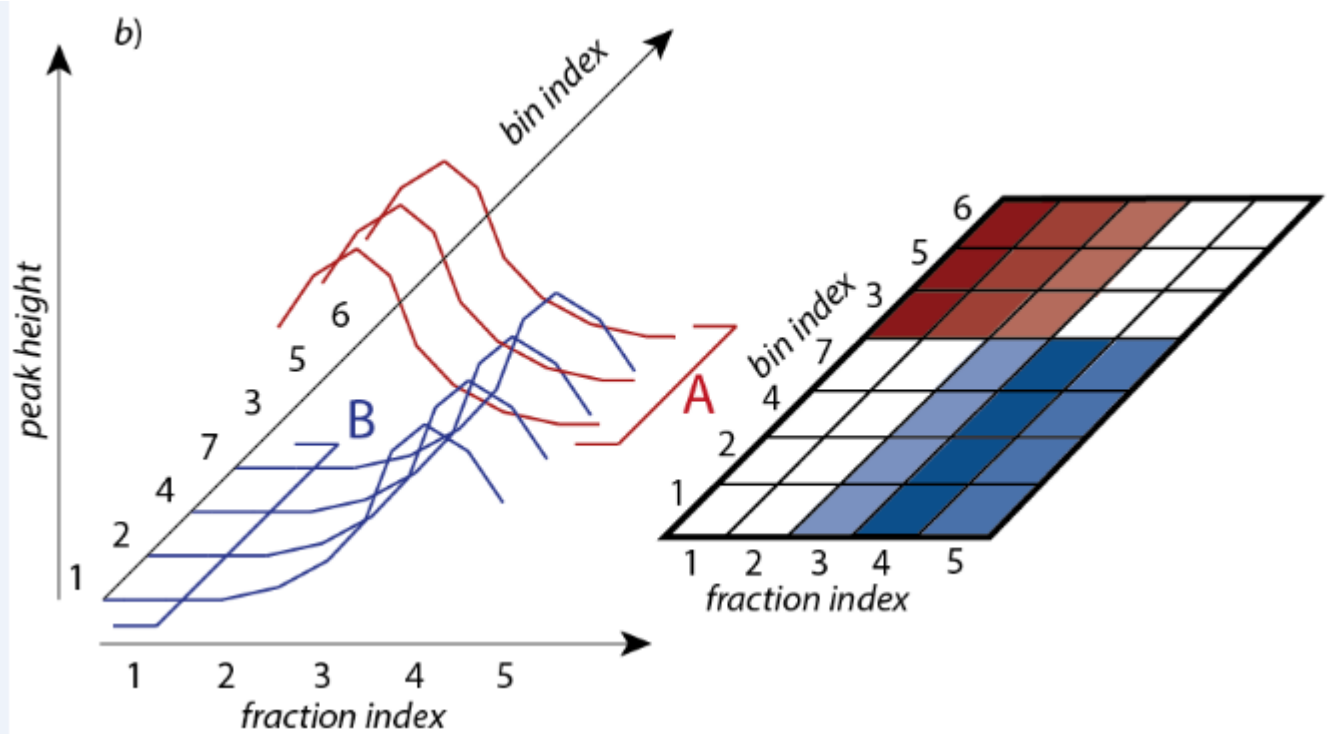
ppm	f1	f2	...	fx
16.3	2E+08	1E+08	...	0
17.5	0	0	...	0
18.7	1E+08	1E+08	...	0
⋮	⋮		Signal intensities	
176.1	3E+07	0	...	0
177.7	0	0	...	4E+07
177.9	6E+07	6E+07	...	0
199.5	7E+07	5E+07	...	0

- Automatic peak picking
- File conversion
- Alignment

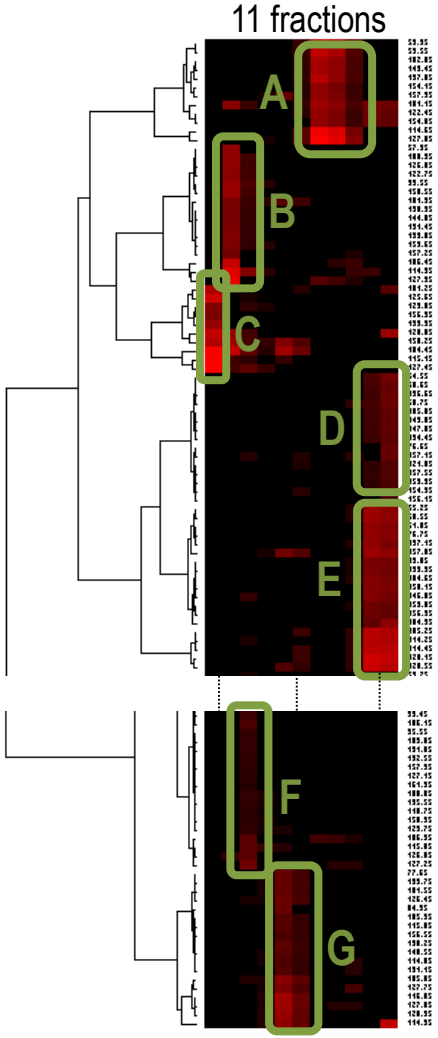
SIMPLIFIED RAW DATA TABLE



REORDERED LINES IN DATA TABLE BY MEANS OF HIERARCHICAL CLUSTER ANALYSIS (HCA)



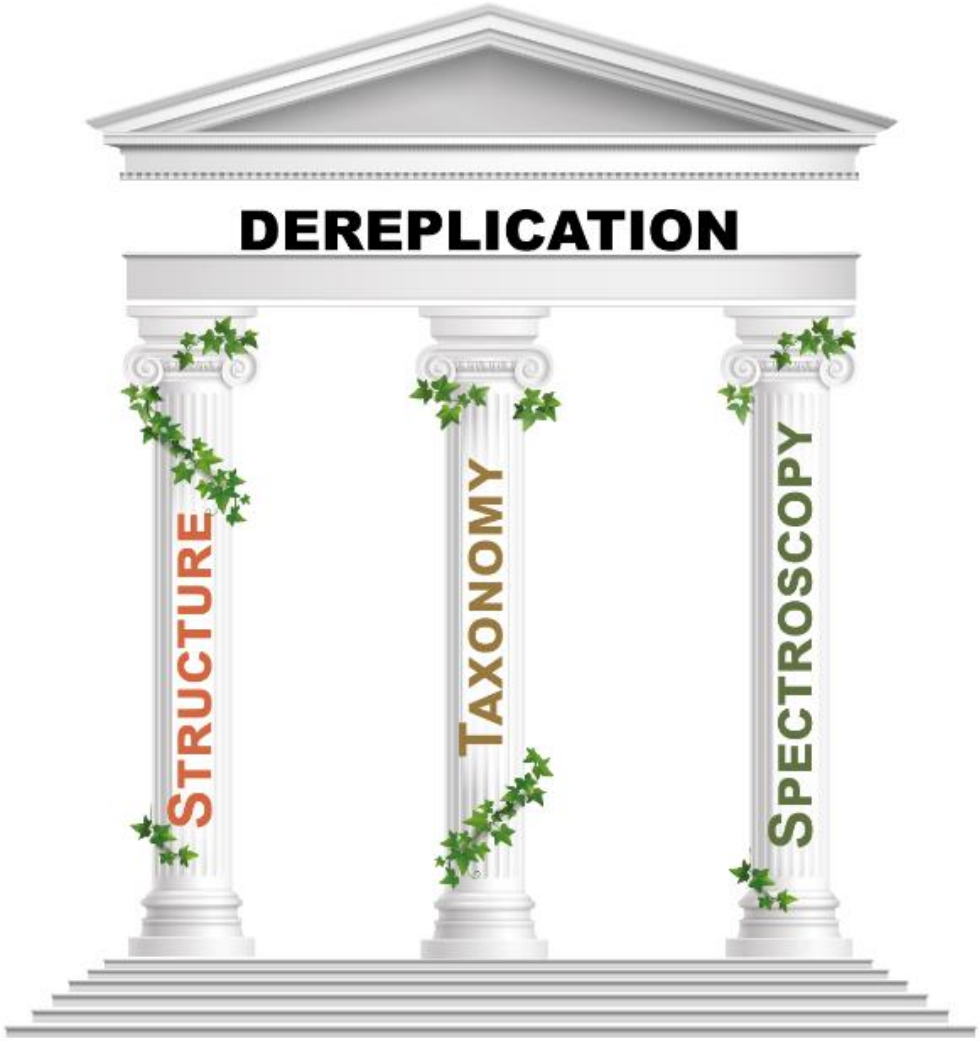
REORDERED LINES IN DATA TABLE BY MEANS OF HIERARCHICAL CLUSTER ANALYSIS (HCA)



} Group of chemical shift values that can be assigned to a **pure compound**

- If a part of the list of chemical shifts is the one related to a known compound, how is it possible to find its structure?
- **The same question holds for a compound that was isolated in pure state.**
- Answering this question requires the availability of *ad hoc* databases.

THE THREE PILLARS OF STRUCTURAL DEREPLICATION



STRUCTURE (+ TAXONOMY)

- Considering only the set of known natural products improves search efficiency (300k?). Where to find them?
- ISDB zenodo.org/record/5607264 About 200k structures + MS
- KNApSAcK www.knapsackfamily.com/knapsack_core/top.php was a first possibility. About 50k compounds. Closed source.
- COCONUT coconut.naturalproducts.net/ has more compounds than KS but not all are natural (407k unique compounds). Open source.
- LOTUS lotus.naturalproducts.net/ is like COCONUT but more selective in its compound selection (276k compounds). It includes natively biological and chemical taxonomies, as well as links to Wikidata www.wikidata.org/wiki/. Open source.

STRUCTURE + ^{13}C NMR

- The **experimental** ^{13}C NMR data are **scattered** in chemical literature, are **not exhaustive**, and sometimes of **low reliability**.
- **Predicted** data are a good alterative to experimental data.
- Prediction requires quick, autonomous, and reliable predictors.
- 200.000 seconds = 2 days, 7 hours, 33 minutes and 20 seconds
- **Solution 1** : nmrshiftdb.nmr.uni-koeln.de with the Java code that allows for predictions locally. Very fast.
- **Solution 2** : www.acdlabs.com ACD/Labs software and
  - "undocumented use" of the experimental chemical shift validation tool (quick, unattended, 2 compounds per second)
  - accurate predictor (slow, GUI-driven, 1 minute per compound).

TWO DEREPLICATION STRATEGIES

- **Initially Untargeted**:
  - The biggest possible collection of structures is associated with predicted spectroscopic data and with taxonomic information. This has to be carried out only at the time of database creation.
  - The results of a structure search can be refined according to taxonomic criteria, if needed.

- **Initially Targeted**:
  - The structures are first selected according to taxonomic criteria. The spectroscopic data are then predicted on demand on a small sized set of structures.
  - Querying a targeted database is quick and provides pertinent structure proposals.

INITIALLY TARGETED STRATEGY

- **KnapsackSearch** (KS) github.com/nuzillard/KnapsackSearch
  - Provide a list of genera related to an organism family to KS
  - Wikipedia is your friend
  - A Python script
    - Queries KNApSAcK through the web about each genus
    - Collects all found structures related to a family
    - Predicts chemical shifts with nmrshiftdb2
    - Creates a .sdf file ACD/Labs software can read
  - Requires an internet connection (slow)
  - Relies on the writing style of data sent back by the KNApSAcK web site (coding in HTML). Will not last forever.
  - Relies on RDKit – https://www.rdkit.org/

INITIALLY TARGETED STRATEGY

- **VersaDB** https://github.com/simremy/versadb_tk
  - Developed by Simon Remy and Julien Cordonnier, @URCA
  - GUI software
  - Compound selection by biological and chemical taxonomy
  - Queries LOTUS through the Internet
  - RMN data prediction by nmrshiftdb2
  - MS^2 data prediction by CFM-ID 4.0
  - Relies on a connection to the Internet
  - Relies on RDKit
  - Should be published soon…

INITIALLY UNTARGETED STRATEGY

- History : SISTEMAT Knowledge Base, Pr. VdP Emerenciano, Brazil
  - Beginning of the1990's.
  - The Three Pillars of Dereplication were already there
  - Closed source databases and software

- « Predicted NMR of Natural Products » (PNMRNP), @ICMR
  - Starting from ISDB and NMR-supplemented using nmrshiftdb2
  - Proof of principle, practical use is not recommended.

INITIALLY UNTARGETED STRATEGY

- **ACD_LOTUS**
  - Structures from LOTUS, supplemented with predicted NMR data
    - Uses ACD/Labs quick validation algorithm for predictions
  - Uses RDKit and the tools developed for KS et PNMRNP
  - 218.478 structures in LOTUS v9
  - zenodo.org/record/7124055 .sdf zipped file
  - Structures and spectra merged into nmrshiftdb2 by Stefan Kuhn
  - The publicly available .sdf file can be imported in ACD/Labs
    - Using acd_lotusv9.NMRUDB does not required a connection to the Internet

EXAMPLE, ACD_LOTUS

- Structure selection of a known specialized metabolite
- Copy the list of the associated ^{13}C NMR chemical shift values
- Look for matching compounds by means of the
  - ACD/Labs database search tool
  - nmrshiftdb2 search tool

EXAMPLE, ACD_LOTUS – ELLIPTICINE

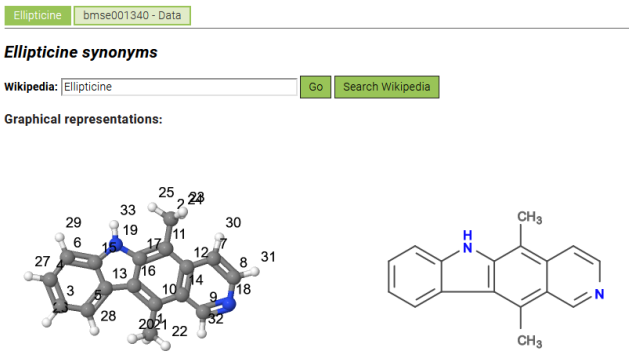
- Ellipticine in the Biological Magnetic Resonance data Bank (BMRB)
- bmrb.io/metabolomics/metabolomics_standards.php?dataset=metabolomics

Filter by dataset NMRFAM highly curated metabolites Go

FAM : Facility at Madison (Wisconsin)

Jump to molecules beginning with: A B C D E F G H I J K L M N O P Q R S T U V X Z

6-ethylmercaptopurine	(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1H-2-benzofuran-5-yl)-4-methylhex-4-enoic acid
(~)-Eburnamonine	EGTA	elaidic acid
Ellagic acid	Ellipticine	Emodin
(-)-Epicatechin	epichlorohydrin	Epinephrine
epsilon-caprolactam	Epsilon-caprolactone	ergocalciferol



FOR WOOD AND NMR LOVERS

- https://bmrb.io/metabolomics/metabolomics_standards.php?dataset=NMR+Database+of+Lignin+and+Cell+Wall+Model+Compounds

Filter by dataset USDA lignin components Go

A description of these data, collected by Sally Ralph and Larry Landucci at the USDA Forest Products Lab, and John Ralph's group at the USDA Dairy Forage Research Center and (more recently) the UW Biochemistry Department, is available as a pdf file. This includes a description of the naming conventions used as well as experimental conditions and a structure index.

Jump to molecules beginning with: A B C D E F G H I L M O P S T V X

A		
1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-(2-methoxyphenoxy)propane	1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-[4-(1-acetoxyethyl)-2,6-dimethoxyphenoxy]propane	1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-[4-(1-acetoxyethyl)-2-methoxyphenoxy]
1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-[4-(1-acetoxyethyl)phenoxy] propane	1-(4-acetoxy-3,5-dimethoxyphenyl)-2-(4-acetoxy-2-methoxyphenoxy)-3-hydroxypropan-1-one	1-(4-acetoxy-3,5-dimethoxyphenyl)-2-(4-acetyl-2,6-dimethoxy phenoxy)-3-hydroxypropan-1-one
1-(4-acetoxy-3,5-dimethoxyphenyl)-2-(4-acetylphenoxy)-3-hydroxypropan-1-one	1-(4-acetoxy-3,5-dimethoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propan-1-one	2-(4-Acetyl-2,6-dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)ethanone

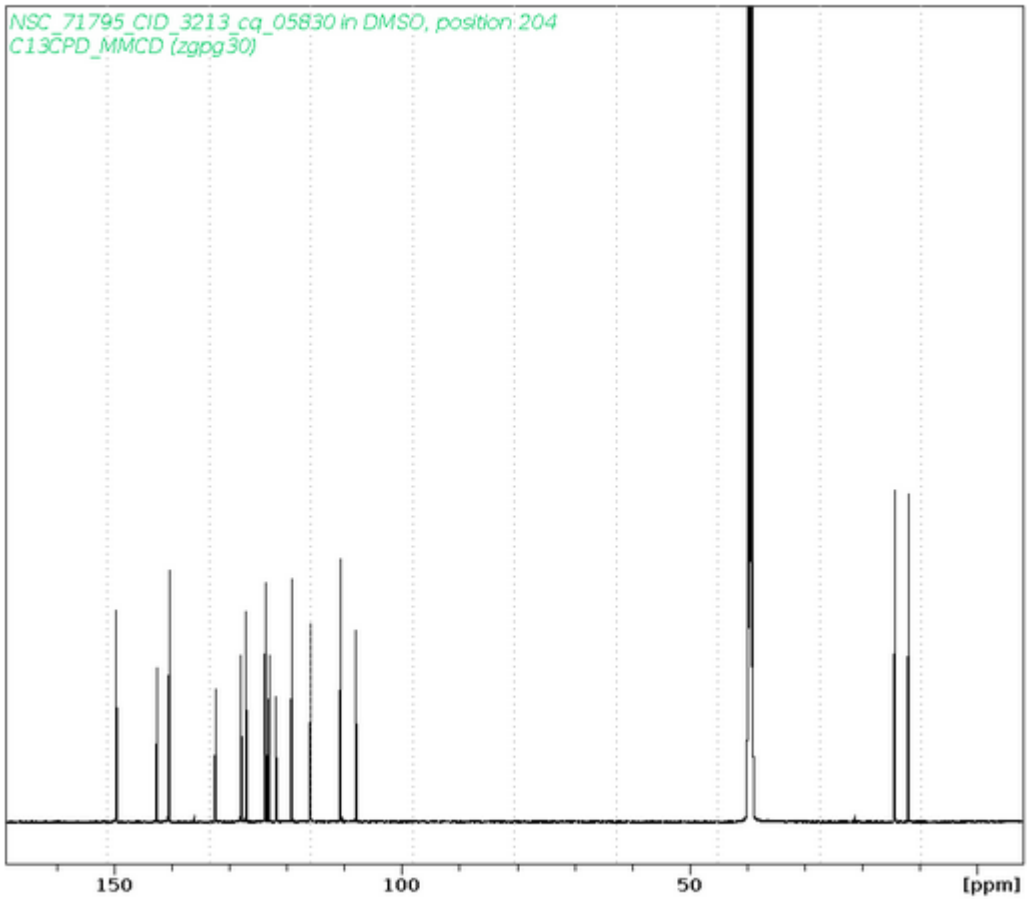
Set 1
Sample: 5mg in DMSO, ref: TMS
Conditions: temperature: 298K
Spectrometer: Bruker Avance III - 500MHz

Atom ID	Value	Ambiguity Code
C1	14.3692	1
C2	11.9824	1
C3	119.191	4
C4	127.1373	4
C5	123.8352	4
C6	110.7067	4
C7	115.8997	1
C8	140.5188	1
C9	149.7257	1
C10	128.066	1
C11	132.4729	1
C12	108.0425	1
C13	123.1352	1
C14	121.9748	1
C15	142.6759	1
C16	123.3902	1
C17	140.5486	1

Experimental values

BACK TO ELLIPTICINE

3: 1D 13C
Sample: 5mg in DMSO, ref: TMS
Conditions: temperature: 298K
Spectrometer: Bruker Avance III - 500MHz



Download time domain data: bmse001340_3.zip

ELLIPTICINE

Query

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127.1373
123.8352
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115.8997
140.5188
149.7257
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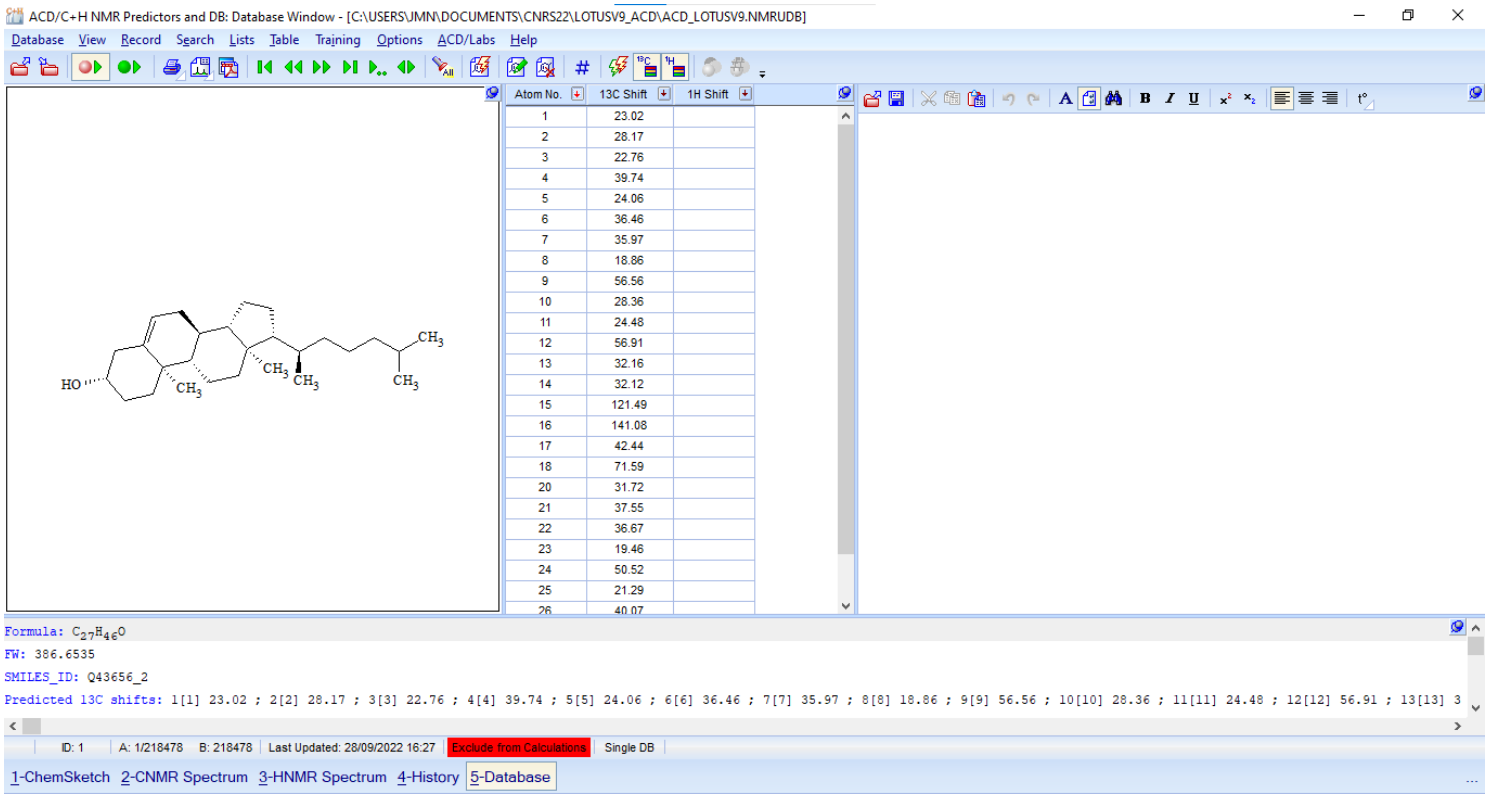
Open file acd_lotusv9.NMRUDB



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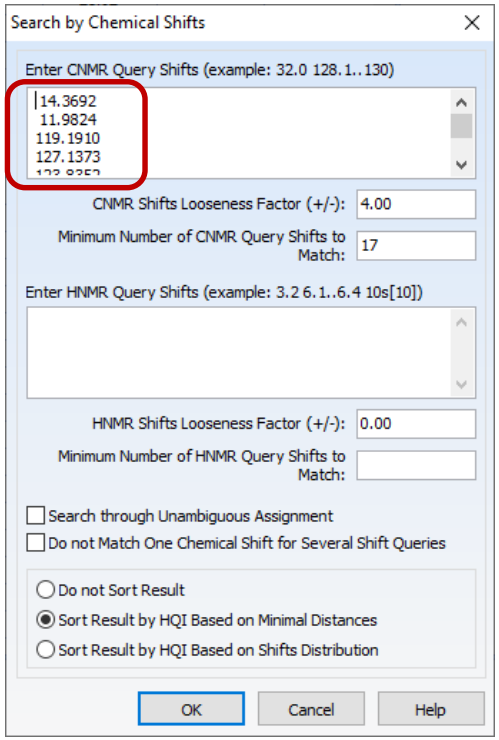

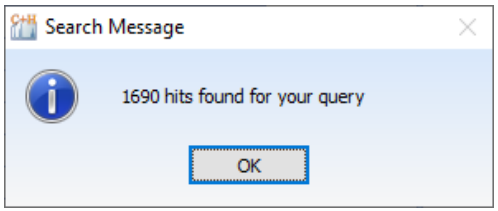

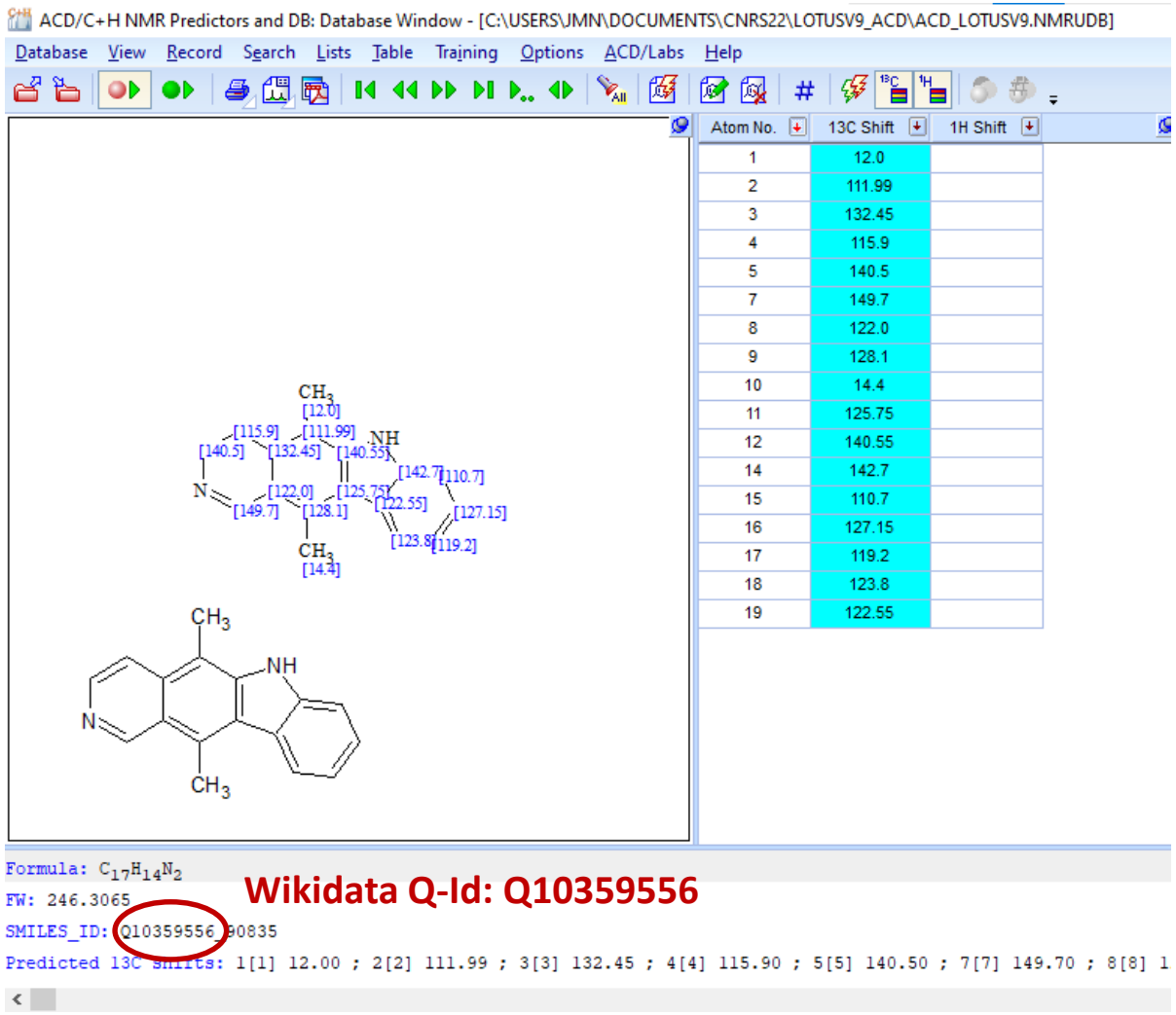

Wikidata Q-Id: Q10359556

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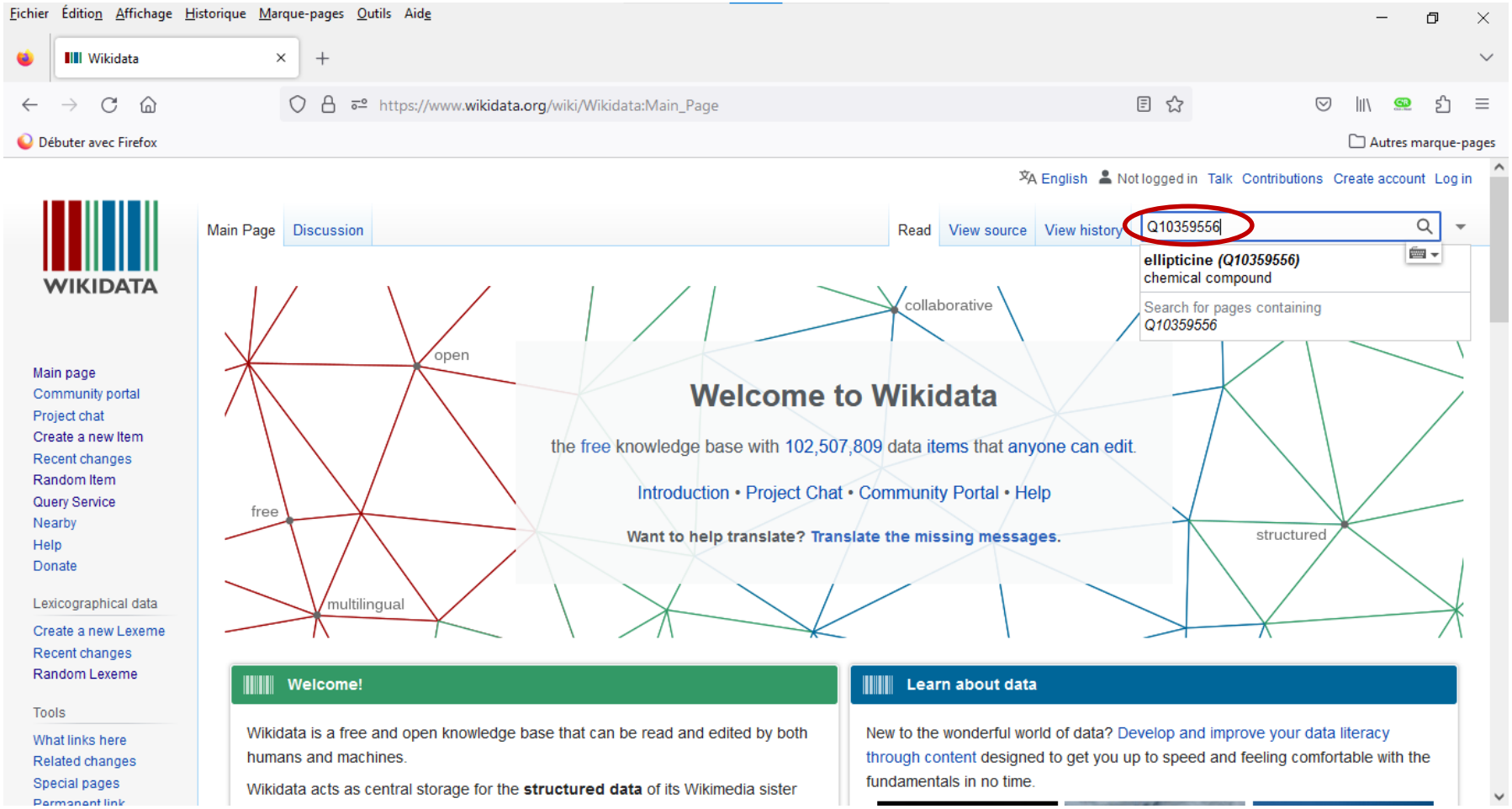
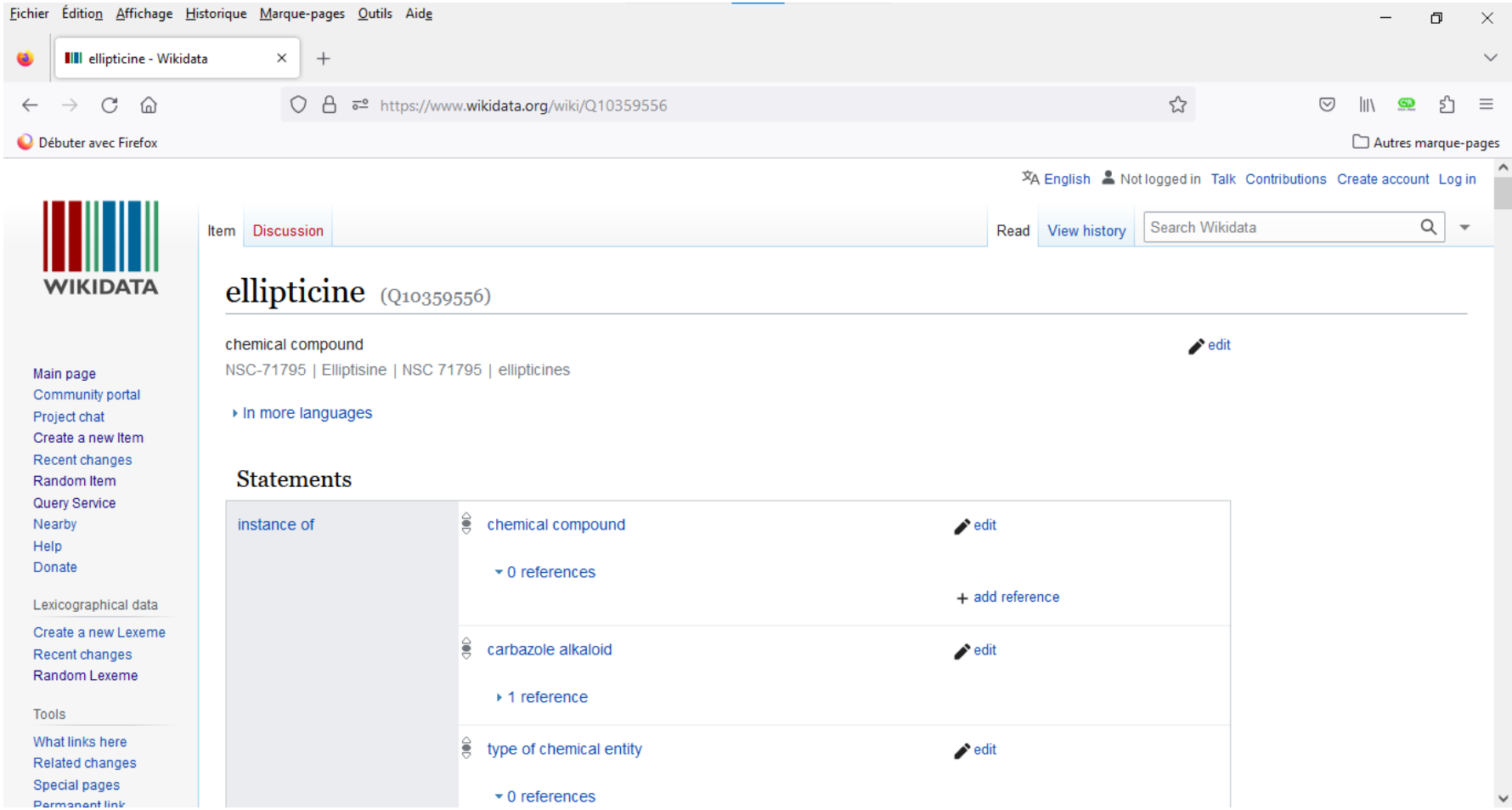


ELLIPTICINE



ELLIPTICINE

found in taxon

Ochrosia elliptica edit

▾ 5 references

stated in	The biogenetic, synthetic and biochemical aspects of ellipticine, an antitumor alkaloid
stated in	Fluorodensitometric assay of 6H-pyrido[4,3-b]-5,11-dimethylcarbazoles (ellipticine and derivatives) biosynthesized by Ochrosia elliptica cultures in vitro
stated in	Antitumor alkaloids in callus cultures of Ochrosia elliptica
stated in	Alkaloid Production by Ochrosia elliptica Cell Suspension Cultures
stated in	Alkaloids of Ochrosia elliptica Labill.1

+ add reference

Ochrosia moorei edit

▸ 1 reference

ELLIPTICINE

WIKIDATA

Item | Discussion

Alkaloids of Ochrosia elliptica Labill.1 (Q104937747)

scientific article published on 10 March 2005

Identifiers

DOI	10.1021/JA01517A031	edit
	0 references	add reference
		add value
		add statement

ELLIPTICINE

Alkaloids of Ochrosia elliptica Labill.[1]

Sidney Goodwin, A. F. Smith, and E. C. Horning

Cite this: C&R J. Am. Chem. Soc. 1959, 81, 8, 1903–1908
Publication Date: April 1, 1959
https://doi.org/10.1021/ja01517a031 C&R
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[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Alkaloids of *Ochrosia elliptica* Labill.[1]

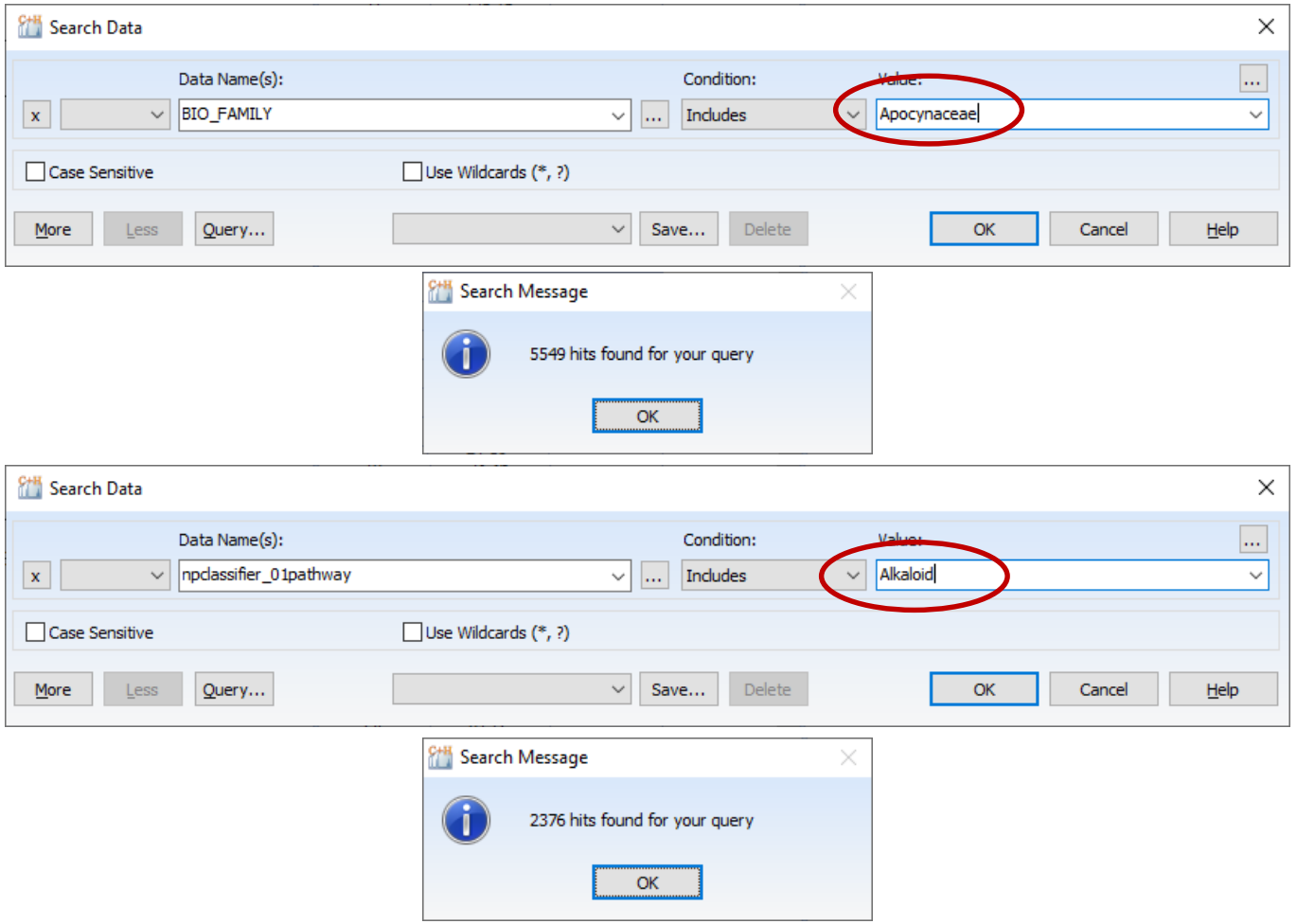
BY SIDNEY GOODWIN, A. F. SMITH[2] AND E. C. HORNING

RECEIVED JULY 28, 1958

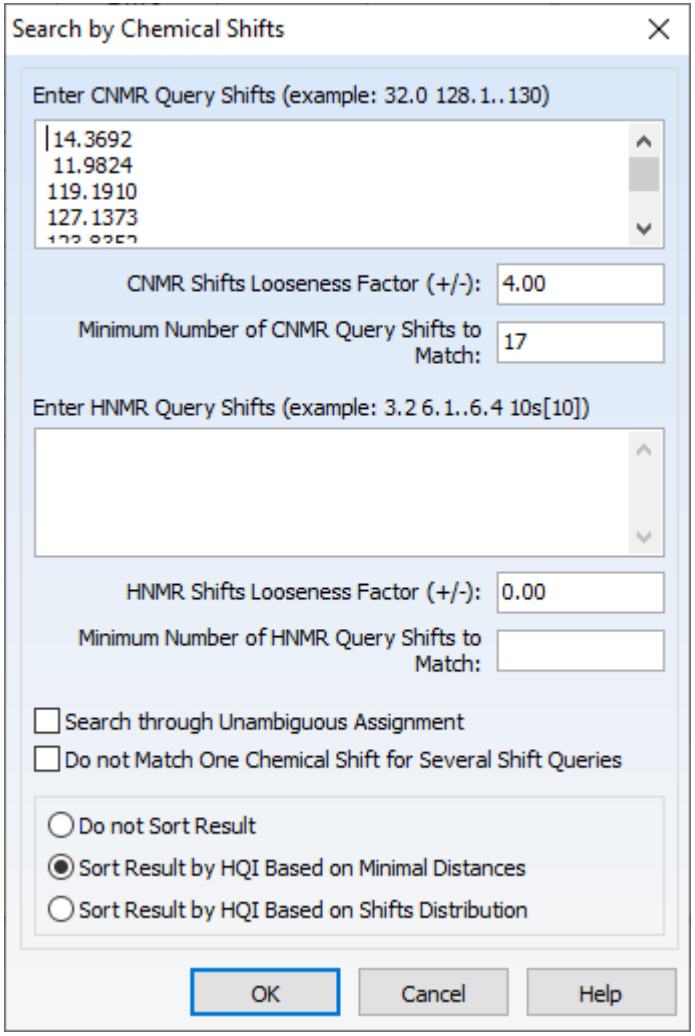
Four alkaloids have been isolated from leaves of *Ochrosia elliptica* Labill. One has been shown to be identical with isoreserpiline. The other three, ellipticine, methoxyellipticine and elliptinine, have been characterized and certain features of their structures have been suggested.

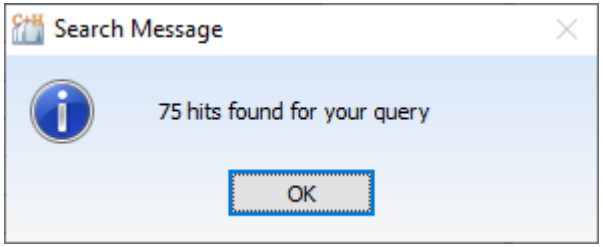
ELLIPTICINE – INITIALLY TARGETED STRATEGY

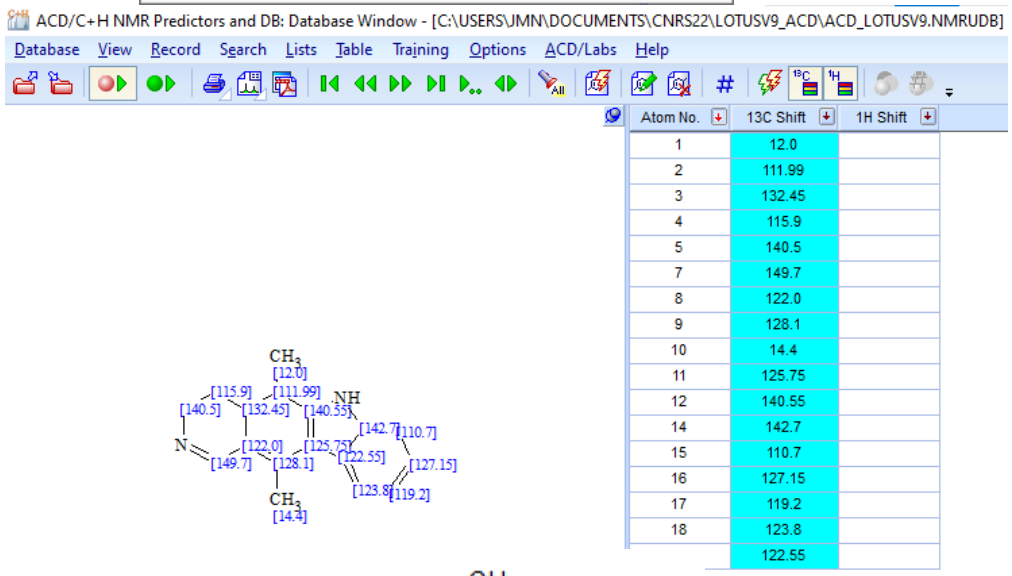
Apocynaceae alkaloids

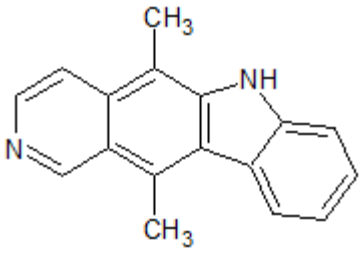


ELLIPTICINE









EXAMPLE, NMRSHIFTDB2 – ELLIPTICINE

Query

14.3692
11.9824
119.1910
127.1373
123.8352
110.7067
115.8997
140.5188
149.7257
128.0660
132.4729
108.0425
123.1352
121.9748
142.6759
123.3902
140.5486

Current usage is:
Registered Users: 2138
Structures: 258158
Spectra: Measured 53954, calculated 396566

Impressum

Home | Search | Results | Quick Check | Predict | Assignment

Search by Spectrum

Switch to expert search mode

Browse all structures

Input list : Input format

```
 14.3692
 11.9824
119.1910
127.1373
123.8352
110.7067
115.8997
140.5188
149.7257
128.0660
132.4729
```

Spectrum type to search:
13C

- ○ Subspectrum
- ◉ Complete

Search by spectrum

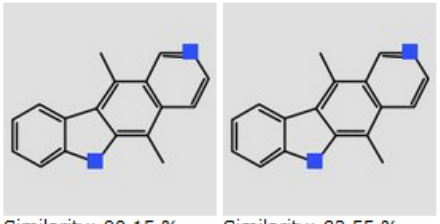
ELLIPTICINE

total similarity spectrum search -- 13C 14.3692|11.9824|119....

Results: 300

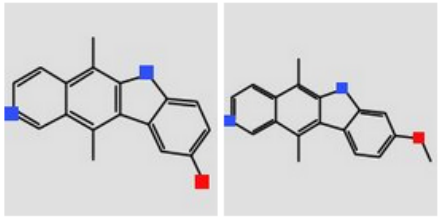
Browse: 1 2 3 4 5 6 7 8 9 10 >>

Next structure



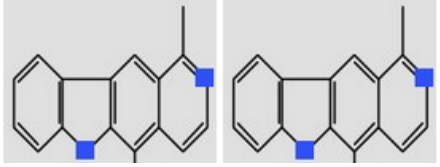
Similarity: 90.15 %

Similarity: 82.55 %



Similarity: 71.02 %

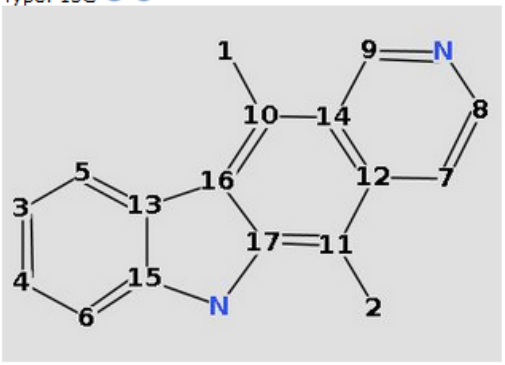
Similarity: 69.05 %

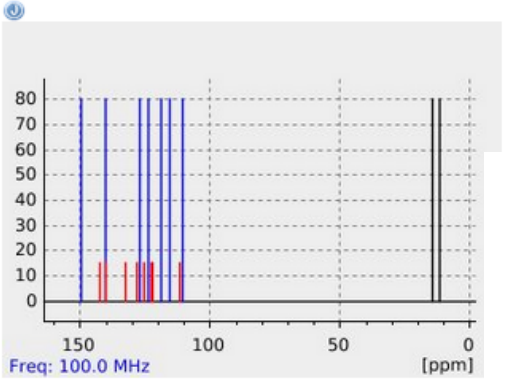


Spectral Data | Additional Data | Download

Search for complete spectrum: Similarity measure for the complete spectrum in this record is 92.92.

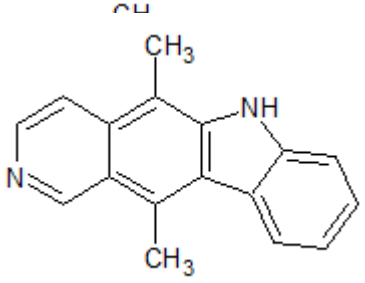
Type: 13C





Atom	Mult.(coupling const.)	Prediction Shift	Input Shift	Diff. M-I	Prediction 0 hose_lotusv7
1	Q	14.40	14.3692	0.03	14.32
2	Q	12.00	11.9824	0.02	11.6
3	D	119.20	119.191	0.01	120.19
4	D	127.15	127.1373	0.01	125.25
5	D	123.80	123.3902	0.41	123.06
6	D	110.70	108.0425	2.66	111.2
7	D	115.90	115.8997	0.00	115.7
8	D	140.50	140.5188	0.02	142.2
9	D	149.70	149.7257	0.03	150.2
10	S	128.10	128.066	0.03	128.34
11	S	111.99	110.7067	1.28	111.0
12	S	132.45	132.4729	0.02	130.38
13	S	122.55	123.1352	0.59	121.95
14	S	122.00	121.9748	0.03	125.6
15	S	142.70	142.6759	0.02	140.95
16	S	125.75	123.8352	1.91	124.83
17	S	140.55	140.5486	0.00	136.36

Threshold is 5.88



TAKE HOME MESSAGE

- The SDF-formatted acd_lotusv9 database is available from https://zenodo.org/record/7124055
- The acd_lotusv9.sdf file can be imported as a searchable database file in ACD/Labs software products
- The structures and chemical shift values from acd_lotusv9 are already searchable in https://nmrshiftdb.nmr.uni-koeln.de/

- The assessment of the known compound identification process was carried out on a small set of 58 secondary metabolites. The results of this study should be published soon.

Natural Products Chemistry Team @ICMR