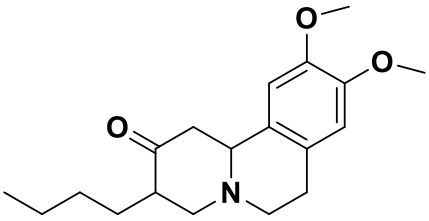
Structure Elucidation Report



Product Name	:	Tetrabenazine Related Compound C
Batch Number	:	SRL-454-139
Mol. Formula	:	C19H27NO3
Mol. Weight	:	317.4 g/mol
CAS No.	:	19328-35-9
CAT No.	:	SZ-T022031
Chemical Name	:	1,3,4,6,7,11b-Hexahydro-9,10-dimethoxy-3-(n-butyl)-2H-benzo[a]quinolizin-2-one

Prepared By	***Reviewed By***	***Approved By***

SYNZEAL RESEARCH PVT. LTD. AHMEDABAD

Brief note on process of confirmation of structure

Elucidation of molecular structure is necessary to identify or confirm the structural identity of a chemical compound during chemical research or product development. The product "Tetrabenazine Related Compound C" has been synthesized and characterised by Research & Development department and Analytical department of SynZeal Research Private Limited.

In the process of structure elucidation various analytical techniques has been performed. For example, Mass spectrometry techniques has been used to determine molecular weight through its m/z ratio. 1H Nuclear Magnetic Resonance spectroscopy and 13C Nuclear Magnetic Resonance spectroscopy used for understanding connectivity of the atoms in the molecule. Whereas Infrared Spectroscopy confirmed the presence of various functional groups. The combination of information from these multiple techniques have given a comprehensive proof of the compound structure and it confirms the identity of the structure.

Following is the list of techniques carried out during structure elucidation and which are briefly explained in following order with its supporting data and methodology -

- IR Spectroscopy
- Mass Spectrometry
- 1H NMR Spectroscopy
- 13C NMR Spectroscopy

The details with spectral data are presented in next pages.

Infrared Spectroscopy

1. ***Details of Instrument***
   - Manufacturer of Instrument – Perkin Elmer
   - Model Name – spectrum 2 (with ATR)
2. ***Analytical Procedure***
   - Approximately weighed 2 mg of compound in moisture free conditions.
   - Cleaned the crystal area of Instrument.
   - After cleaning crystal area, background spectrum of empty crystal area has been recorded.
   - After collection of background spectrum, compound has been placed on the crystal area.
   - After that solid has been placed on the crystal area, then after the pressure arm has been positioned over the sample area.
   - Then after spectra have been recorded for sample.
3. ***Observation***
   - The spectra have been recorded in the range of 4000 cm^{-1} to 400 cm^{-1} with 8 scans. Following is the observation table which has been acquired from IR Spectroscopy.

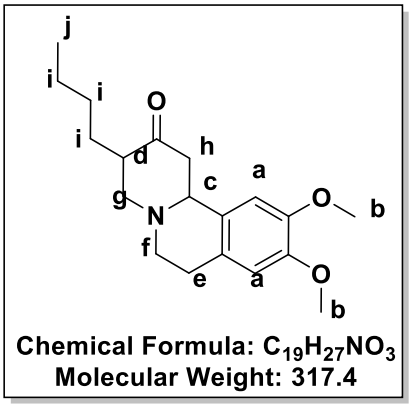
Functional Groups	Wave Number (cm^{-1})
C=C(Aromatic)	1609.63
C=O	1701.83
C-N	1293.82

4. Conclusion

The signals of the IR spectrum and their interpretation are consistent with the structural formula.

^{1}H Nuclear magnetic resonance spectroscopy

1. ***Details of Instrument***
   - Manufacturer of Instrument – Bruker
   - Model Name – 400 MHz
2. ***Analytical Procedure***
   - Approximately weighed 5 mg of compound in moisture free conditions.
   - Dissolved sample in appropriate deuterated solvents.
   - After that small amount (~0.1%) of reference compound Tetramethylsilane (TMS) added in the sample.
   - Sample solution has been filtered into the sample tube through a Pasteur pipette containing an oven dried glass wool plug.
   - After the sample preparation, the sample tube has been placed in the spinner and the spinner in the depth gauge.
   - After that the depth gauge has been removed before inserting the sample and spinner into the magnet.
   - Then after spectra have been recorded for sample.
3. ***Observation***
   - The 1H NMR spectra has been recorded in DMSO-D6 and data has been classified in the following table.



Chemical Formula: $C_{19}H_{27}NO_3$
Molecular Weight: 317.4

Proton Assignment	Chemical Shift δ	No. of Proton
a	6.684-6.698	2
b	3.700-3.725	6
c	3.438	1
d	3.160-3.203	1
e	2.841-3.031	2
f	2.773-2.824	2
g	2.404-2.592	2
h	2.138-2.174	2
i	0.964-1.689	6
j	0.707-0.743	3

4. Conclusion

- The structure is confirmed with the signals of the spectrum and their interpretation.

13C Nuclear magnetic resonance spectroscopy

1. *Details of Instrument*

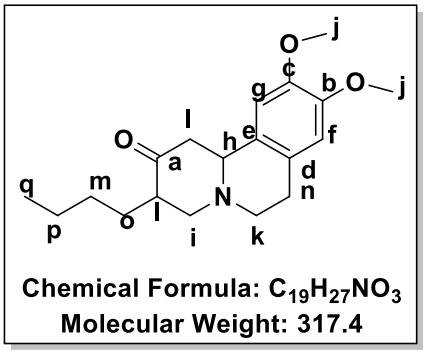
- Manufacturer of Instrument – Bruker
- Model Name – 400 MHz

2. *Analytical Procedure*

- Approximately weighed 15 mg of compound in moisture free conditions.
- Dissolved sample in appropriate deuterated solvents.
- After that small amount (~0.1%) of reference compound Tetramethylsilane (TMS) added in the sample.
- Sample solution has been filtered into the sample tube through a Pasteur pipette containing an oven dried glass wool plug.
- After the sample preparation, the sample tube has been placed in the spinner and the spinner in the depth gauge.
- After that the depth gauge has been removed before inserting the sample and spinner into the magnet.
- Then after spectra have been recorded for sample.

3. *Observation*

- The 13C NMR spectra has been recorded in DMSO-D6 and data has been classified in the following table.



Chemical Formula: $C_{19}H_{27}NO_3$
Molecular Weight: 317.4

Carbon Assignment	Chemical Shift δ	No. of Carbon
a	212.130	1
b	147.784	1
c	147.527	1
d	128.024	1
e	127.107	1
f	112.171	1
g	108.551	1
h	65.226	1
i	56.123	1
j	55.747-55.148	2
k	54.836	1
l	51.214	2
m	29.494	1
n	29.136	1
o	26.192	1
p	22.075	1
q	14.208	1

4. Conclusion

- The structure is confirmed with the signals of the spectrum and their interpretation.

Mass spectrometry

1. Details of Instrument

- Manufacturer of Instrument – Shimadzu
- Model Name – LCMS-2020

2. Analytical Procedure

- Approximately weighed 2 mg of compound in moisture free conditions.
- Dissolved sample in appropriate solvent and approximately 2mL of solution having the concentration of 200 ppm have been made and filtered it.
- Transferred the prepared solution into the vial and placed the vial in autosampler.
- After that appropriate Ionization probe (ESI/APCI) adjusted in Instrument.
- Minimum amount of sample injected in Instrument through autosampler and the spectra has been recorded.

3. Observation

- The Mass spectra has been recorded in Acetonitrile and data has been classified in the following table.

Sr. No.	M/Z	Fragments
a	318.19	M + 1

4. Conclusion

- The signals of the mass spectrum and their interpretation are consistent with the structural formula.