Available online on 15.05.2022 at *http://ujpr.org*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)
Open access to Pharmaceutical Research

Volume 7, Issue 2, 2022

RESEARCH ARTICLE

A NOVEL QUATERNARY ALKALOID *N*-METHYLCORYXIMINE FROM *HYPECOUM PENDULUM* L.

Ergün METE, Hüsniye Kayalar*
Ege University, Faculty of Pharmacy, Department of Pharmacognosy, Bornova, İzmir- Türkiye.

ABSTRACT

Objective: In this study, the isolation and chemical structure elucidation of a new alkaloid from *Hypecoum pendulum* L is provided.

Material and Methods: Column and ascending preparative thin layer chromatographic methods were utilized for the purification of the novel compound. The structure elucidation was determined based on spectroscopic methods including infrared (IR), nuclear magnetic resonance (NMR), and Mass (MS) spectroscopy.

Results: *N*-methylcoryximine, a quaternary alkaloid from secoberbine subgroup of isoquinoline alkaloids, was isolated and the structure of this compound was elucidated.

Conclusion: The natural occurrence and spectral data of *N*-methylcoryximine is reported for the first time.

Keywords: *Hypecoum pendulum*; N-methylcoryximine; quaternary alkaloid.

Article Info: Received 7 March 2022; Revised 3 April; Accepted 5 May, Available online 15 May 2022

Cite this article-
METE E, Kayalar H. A novel quaternary alkaloid N-methylcoryximine from *Hypecoum pendulum* L. Universal Journal of Pharmaceutical Research 2022; 7(2):40-47.

DOI: *https://doi.org/10.22270/ujpr.v7i2.746*

Address for Correspondence:
Dr. Hüsniye Kayalar, Ege University, Faculty of Pharmacy, Department of Pharmacognosy, Bornova, İzmir-Türkiye. Tel- +902323112549; E-mail: *husniye.kayalar@ege.edu.tr*

INTRODUCTION

The plant genus *Hypecoum* (Papaveraceae), represented by 15 species, is distributed from Mediterranean to Central Asia and Northern China[1]. *Hypecoum* species are traditionally used as analgesic in Russia[3]. In Tibetian medicine, plants from this genus are used as analgesic, anti-inflammatory and antipyretic[4]. *Hypecoum* genus is a rich source for isoquinoline alkaloids[5-8]. Research performed on *Hypecoum* species has revealed a number of pharmacologically active alkaloids. Allocryptopine, corydamine, cryptopine, hypecorine, leptocarpinine, protopine from *Hypecoum* species exerted numerous potential biological activities such as antibacterial, antitumoral, and anti-inflammatory[9-12].

H. pendulum L. is an annual herb with erect stems ascending up to 30 cm. The linear leaves with short hairs are pinnatisect and 7, 8-10,9 cm long. The two yellow exterior petals are entire, oval-rhomboid and 0.52-0.76 cm long. The pendulous fruits are 4.20-5.75 cm long and, borne on a short, recurved pedicel, straight, attenuate at apex, weakly striate and lomentoid[13-14]. Following the search on *H. pendulum*, the isolation of protopine, 8-methoxy dihydrosanguinarine, oxysanguinarine, oxyhydrastine, stylopine, turkiyenine, dihydrosanguinarine, hypecorine, (+)-oxoturkiyenine, bulbocapnine, glaucine, corydalisol were previously reported[15].

The present study deals with further investigation for the quaternary alkaloidal content of *H. pendulum*. To the best of our knowledge this is the first report on the isolation and structure elucidation of a new quaternary alkaloid *N*-methylcoryximine.

MATERIALS AND METHODS

Plant material

H. pendulum L. (Papaveraceae) was collected from Usak-Gediz-Izmir road, and the voucher specimen No. 1031 was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Izmir Türkiye. Plant was identified by one of the authors, Dr. Ergün Mete.

Extraction and purificaton

The whole parts of *H. pendulum* were collected, air dried and pulverized by mill (Retsch Gmbh SK 1). The powdered plant material (13.18 kg) was extracted with 96% ethanol (116 L) by maceration under continuous shaking. Briefly, the coarsely powdered plant material was placed inside a bottle and the menstruum is poured on top until completely covered the drug material. The bottle is then closed and kept for two days. Then the bottle was shaken time to time to ensure complete extraction. At the end of extraction, the micelle was separated from marc by filtration. The solvent was evaporated in vacuum to obtain crude ethanolic extract (3.85 kg). The crude ethanolic extract was acidified with 5% aqueous hydrochloric acid (38.8 L). The resulting aqueous acidic solution was extracted with petroleum ether (14 L) to remove resins and lipids. The defatted aqueous acidic solution was adjusted with hydrochloric acid to obtain 3% aqueous hydrochloric acid solution. The acidic solution was extracted with chloroform (60 L). After the evaporation of the organic solvent under vacuum, the acidic extract (12.49 g) was chromatographed over column prepared by silica gel 60 H (70-230 mesh), employing chloroform and increasing amounts of methanol. Total 500 ml of each fraction were collected. The monitoring was determined by thin layer chromatography and similar fractions were combined. The residue from 25-29 fractions was subjected to ascending preparative thin layer chromatography on silica gel glass plates using chloroform: methanol (7:3). The bands were differentiated under short wavelength ultraviolet light and by means of Dragendorff's reagent. The bands were desorbed from silica gel using the solvent system $CHCl_3$-MeOH (50:50 with descending method[15-16]. The compound given the code as EVM-1 was obtained as an amorphous white powder (1.94 g).

Experimental procedures

General

Solvents used in the extraction and purification process, TLC precoated silica gel 60 F_{254} plates and silica gel 60 H (70-230 mesh) used for preparative column chromatography were obtained from Merck. Specific rotation of the compound in methanol was measured on a Perkin-Elmer 241 polarimeter. Spectroscopic methods were used to determine the structure of the alkaloid.

Infrared (IR) spectrum

IR spectrum was taken by preparing pellets of the compound in potassium bromide in JASCO FT/IR-430 Infrared spectrophotometer.

Nuclear Magnetic Resonance (NMR) Spectrum

^{1}H NMR spectrum, ^{13}C NMR, Distortionless Enhancement by Polarization Transfer Spectroscopy (DEPT) and two dimensional NMR (2D NMR) experiments including ^{1}H-^{1}H Double Quantum Filtered Correlation Spectroscopy (DQF COSY), ^{1}H-^{13}C Heteronuclear Single Quantum Coherence (HSQC), Total Correlation Spectroscopy (TOCSY), Heteronuclear Multiple Bond Correlation (HMBC) and Rotating Frame Overhauser Enhancement Spectroscopy (ROESY) were taken in DMSO on a Bruker AMX-600 spectrophotometer.

Mass Spectrum

Electrospray ionization mass spectra (ESI) was acquired on Finnigan MAT TSQ 700 spectrometer.

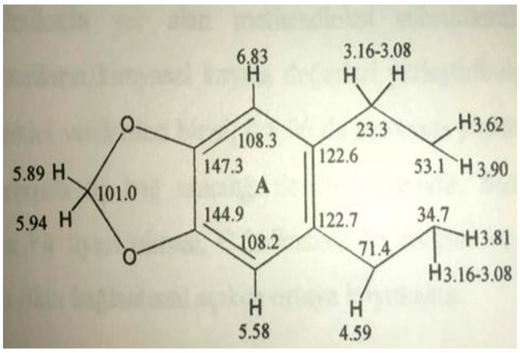


Figure 1: Methylenedioxy group and hydrogens with two *para*-positions, a triple and a quadripol spin system bonded at ortho positions.

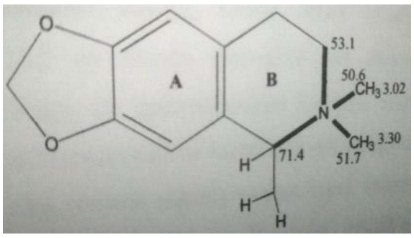


Figure 2: Methyl groups on Nitrogen atom.

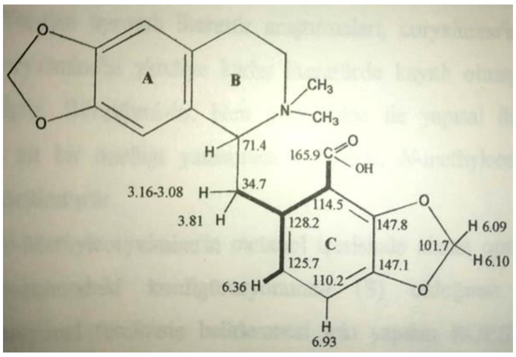


Figure 3: Carbonyl residue, geminal aliphatic protons and aromatic protons on the ring C.

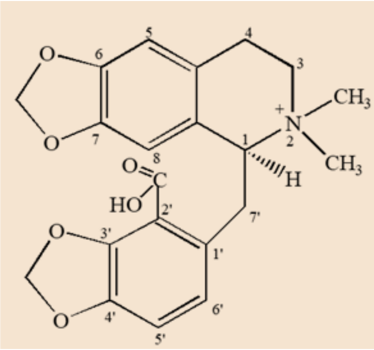
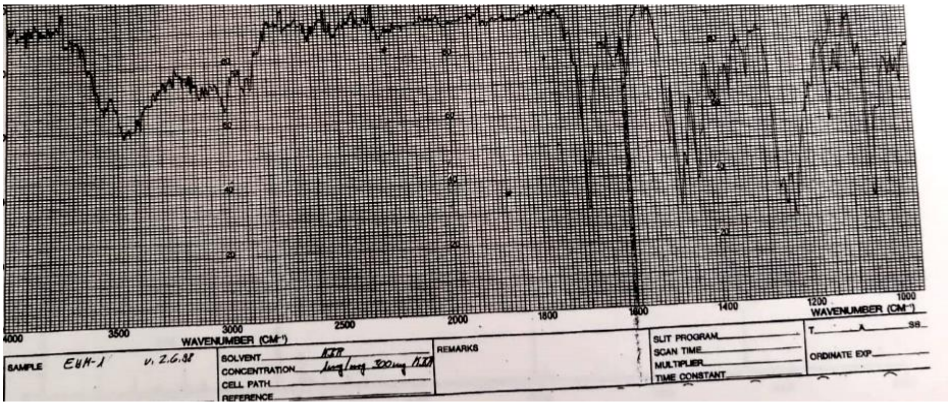


Figure 4: *N*-methylcoryximine.



Spectrum 1: IR spectrum of *N*-Methyl-coryximine.

RESULTS AND DISCUSSION

Spectral data

The spectral data of the compound is as follows:

$(\alpha)_D$ (MeOH, c 0.1) -0.105^0

IR (KBr): 3485, 3005, 1690, 1490, 1235, 1065, 925 cm^{-1}

^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.93 (1 H, *d*, *J*=8.0 Hz, H-5′), 6.83 (1H, s, H-5), 6.36 (1H, *d*, *J*=8.0 Hz, H-6′), 6.10 (1H, s, OCH_2O), 6.09 (1H, s, OCH_2O), 5.94 (1H, s, OCH_2O), 5.84 (1 H, s, OCH_2O), 5.58 (1H, s, H-8), 4.59 (1H, *dd*, *J*=4.2, 10.5 Hz, H-1), 3.90-3.85 (1H, m, H-3), 3.81 (1H, *dd*, *J*=4.3, 12.5 Hz, H-7′), 3.62 (1H, *dd*, *J*=7.4, 13 Hz, H-3), 3.16-3.08 (3 H, m, H-4, H-7′), 3.02 (3 H, s, *N*-CH_3); 3.30 (3 H, s, *N*-CH_3)

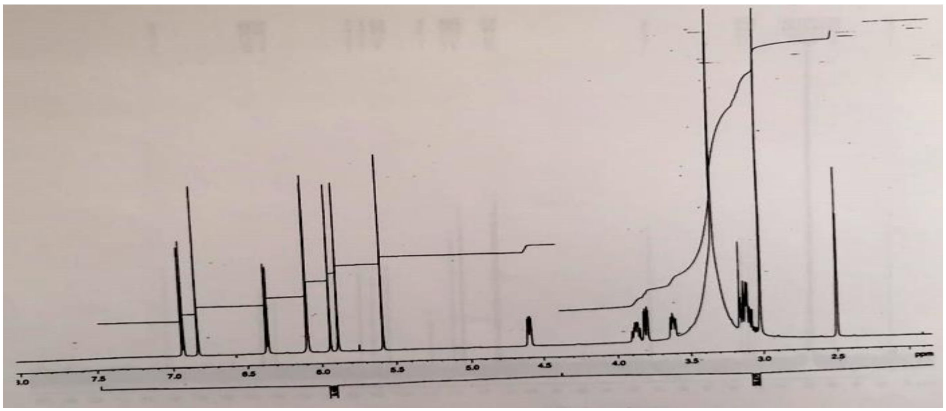
^{13}C-NMR (150 MHz, DMSO-d_6): δ 23.3 (C-4), 34.7 (C-7′), 50.6 (*N*-CH_3), 51.7 (*N*-CH_3), 53.1 (C-3), 71.4 (C-1), 101.0 (OCH_2O), 101.7 (OCH_2O), 108.2 (C-8), 108.3 (C-5), 110.2 (C-5′), 114.5 (C-2′), 122.6 (C-4a), 122.7 (C-8a), 125.7 (C-6′), 128.2 (C-1′), 144.9 (C-7), 147.1 (C-4′), 147.3 (C-6), 147.8 (C-3′), 165.9 (C=O)

2D NMR spectrums ^{1}H-^{13}C NMR, DEPT, ^{1}H-^{1}H DQF COSY, HSQC, HMBC, ROESY and TOCSY,

ESI-MS m/z (rel. int%): 384 $[M]^+$ (100). Calcd. For $C_{21}H_{22}NO_6$

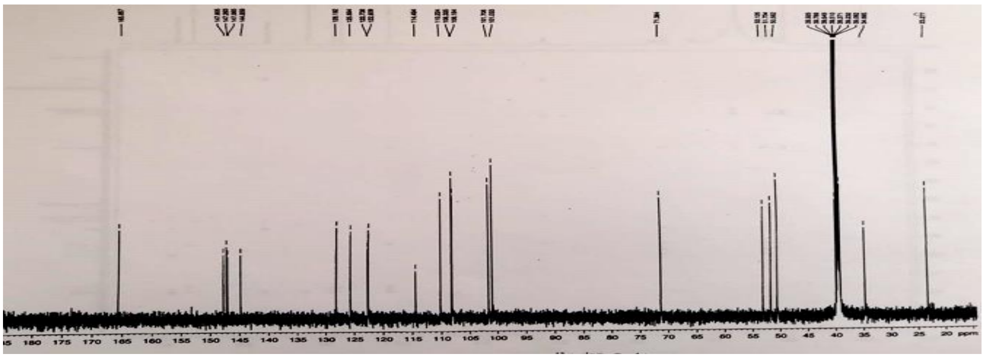
Structure elucidation

The compound EVM-1 from *H. pendulum* extract was obtained by column chromatography fractionation and purified by preparative thin layer chromatography. This compound gave a positive alkaloid reaction with Dragendorff's spray reagent. The optical rotation of the compound was measured by polarimeter and it was found to be levorotatory.



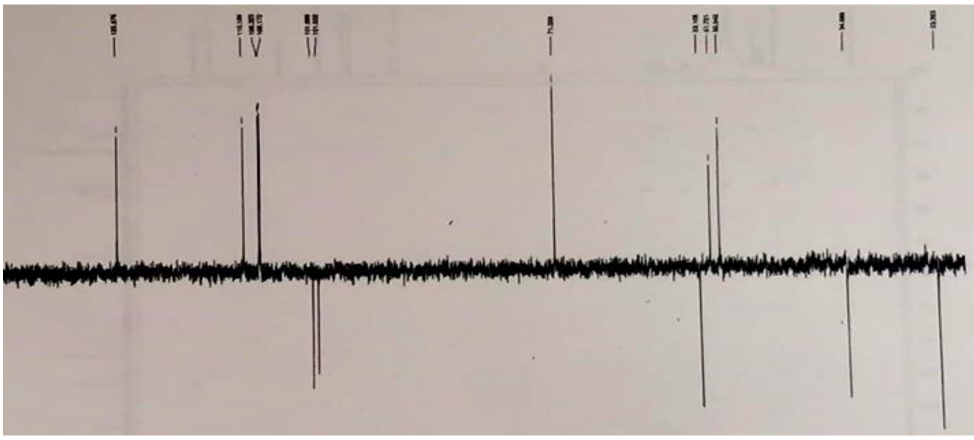
Spectrum 2: ^{1}H NMR spectrum *N*-Methyl-coryximine.

The ^{1}H NMR spectrum in dimethyl sulfoxide (DMSO) displayed two doublets (*J*=8.0 Hz), appearing at δ= 6.93 and δ=6.36 ppm in the aromatic field, indicated the presence of two hydrogens with ortho substitution to each other. One singlet proton was also observed at δ=6.83 ppm in the aromatic region. In the high frequency region, sharp singlets with one proton each were observed at δ=6.10, 6.09, 5.94 and 5.89 ppm suggested that these signals may belong to two methylenedioxy groups. The signal observed at δ= 5.58 ppm seemed to be at upper field for aromatic hydrogen. The most striking sharp singlet of proton, observed at δ=3.02 ppm in the aliphatic field, suggested the presence of a downfield-shifted *N*-methyl group due to its chemical environment. In addition to this, seven proton signals, almost all of which have shifted downfield, were observed. The spin-spin coupling constants of doublets at δ= 4.59, 3.81 and 3.62 ppm were easily determined. In contrast, the coupling constants of the multiplet signals, accounting for the presence of three protons at δ=3.90-3.85 ppm and at δ=3.16-30.8 ppm, were undetectable. The ^{1}H NMR data provided the presence of 18 protons. The multiplicities of the 21 carbons accounted for in the ^{13}C NMR spectrum of EVM-1 were determined by a Distortionless Enhancement by Polarization transfer (DEPT) experiment.



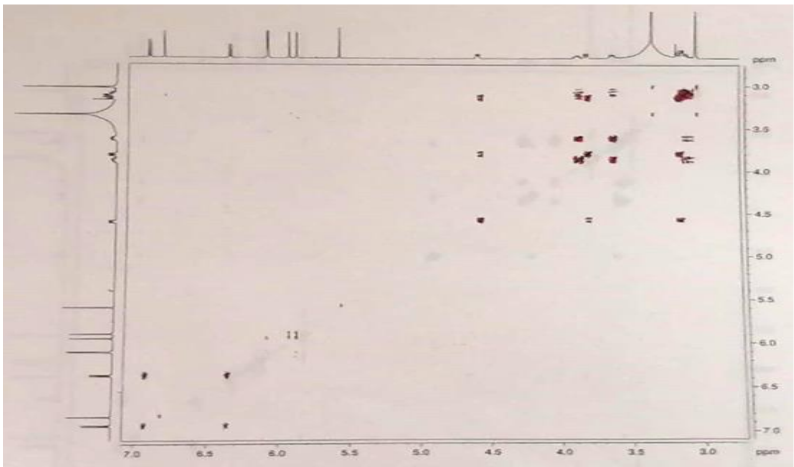
Spectrum 3: ^{13}C NMR spectrum of *N*-methylcoryximine.

Explicit assignments of all carbon chemical shifts were achieved by HSQC and HMBC experiments. The resonance of carbon at δ=165.9 ppm, indicated the presence of a carbonyl group in the compound. Additionally, this was supported by the absorption band in its IR spectrum at 1690 cm^{-1}. In the same DEPT spectrum, signals at δ=101.2 and 101.3 ppm confirm the two methylenedioxy substituents.

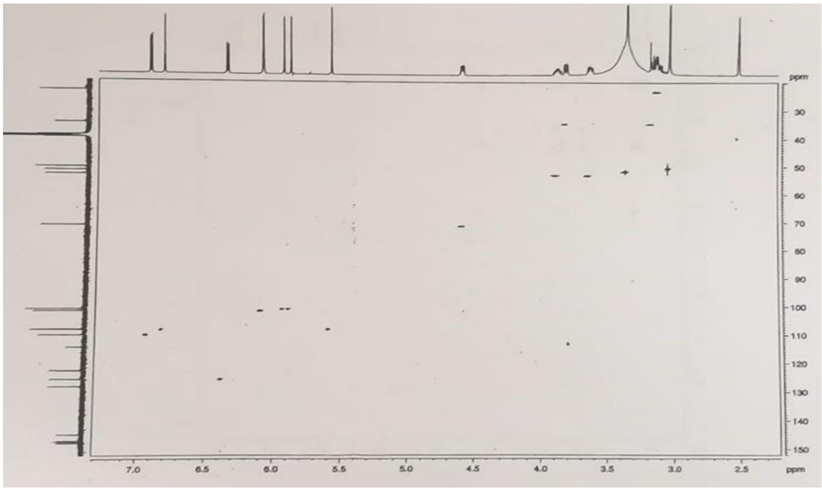


Spectrum 4: DEPT spectrum of *N*-methylcoryximine.

It was not possible to definitely judge about whether the signal at δ=51.7 ppm belongs to the methyl or methine carbon. Also, a corresponding proton signal was not observed at ^{1}H NMR spectrum. In this case, both spectra were carefully evaluated again and it was concluded that the hydrogens on this carbon might be masked by the broad and strong methanol solvent signal observed at δ=3.30 ppm in the ^{1}H NMR spectrum. To clarify the structure, double-dimension NMR experiments (H-1H DQF COSY, HSQC, TOCSY, HMBC and ROESY) were performed on the compound. Protons on the carbons were paired with the HSQC experiment giving $^{1}J_{CH}$ correlations. In this context, it revealed that the two protons located in the upper field with δ=3.16-3.08 ppm were geminal protons and they were bound to the carbon signaling at δ=23.3 ppm. The third proton located in the lower field of the same cluster was geminal with the proton signaling at δ=3.81 ppm which was located on the carbon at δ=34.7 ppm.

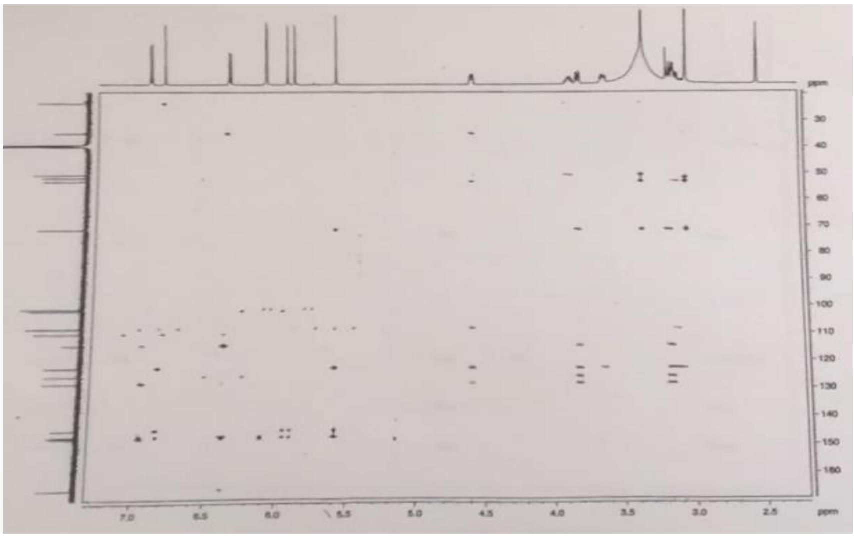


Spectrum 5: 1H-1H DQF COSY spectrum *N*-methylcoryximine.



Spectrum 6: HSQC spectrum *N*-methylcoryximine.

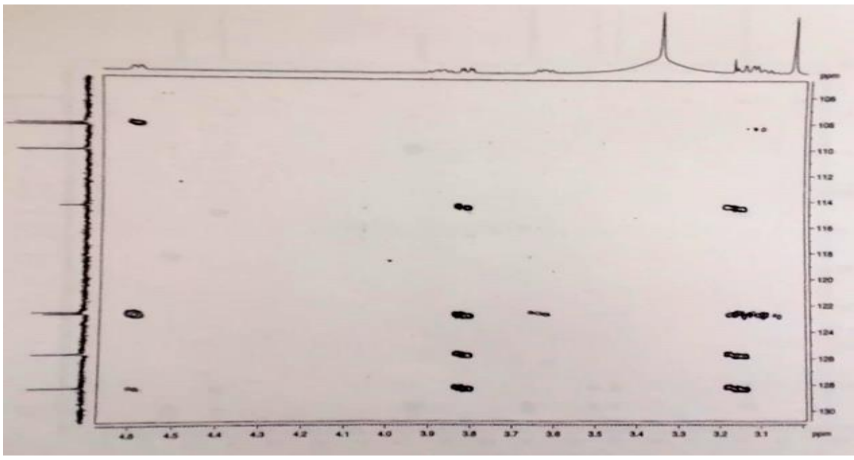
The protons forming the third geminal pair were observed at δ=3.90-3.85 and 3.62 ppm. This methylene group was located on the carbon signaling at δ=53.1 ppm. It was certainly been demonstrated that the proton located at the carbon (δ=51.7 ppm) was indeed masked by signal observed for methanol solvent (δ=3.30 ppm). However, it was not possible to make a judgement as to whether this signal corresponds to methine or methyl protons. In the ^{1}H-^{1}H DQF COSY experiment, it was observed that the methylene group protons signaling at δ=3.81 ppm and at δ=3.16-3.08 ppm in the lower area of the cluster formed a triple-spinned system with proton at δ=4.59 ppm.



Spectrum 7: HMBC spectrum *N*-methylcoryximine.

In the same spectrum, it was observed that the methylene group, consisting of two protons signaling in the upper field of the δ=3.16-3.08 ppm signal cluster, forms an isolated quadripolar spin pairs with the methylene protons at δ=3.62 and 3.90 ppm. The existence of these systems was confirmed by the data obtained from the TOCSY experiment. The most important information describing the structure of the compound was obtained from the HMBC spectrum showing the $^2J_{CH}$ and $^3J_{CH}$ correlations. Evaluation of this spectrum was started with the signals belonging to the protons, which were observed as singlets and therefore predicted to be in *para* position on the aromatic ring. The interaction of carbons with protons (δ=6.83 and 5.58 ppm) via two and three bonds were determined. Thus, ring A was proposed to have a 1,2,4,5-tetra substituted benzene structure. In addition, there was a methylenedioxy group and protons with two *para*-positions, as well as a triple and a quadripololar spin system bonded at *ortho* positions (Figure 1).

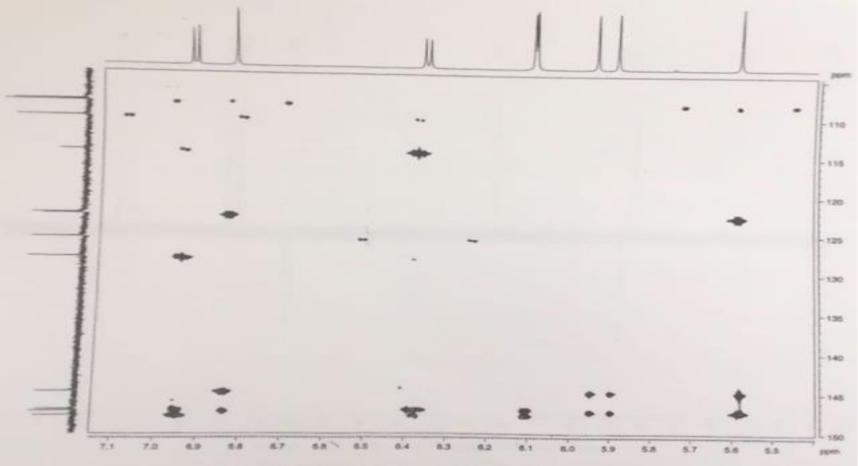
During the interpretation of one-dimensional NMR experiments, it was predicted that the three singlets observed at δ=3.02 ppm in ^{1}H NMR spectrum could be the *N*-methyl group protons shifting down to the lower field due to its chemical environment. The fact that these protons, exhibiting the same $^4J_{CH}$ correlations with the three carbons signaling at δ=53.1, 71.4 and 51.7 ppm, provided conclusive evidence for ring B and for elucidating the nature of not fully resolved carbon signal at δ=51.7 ppm. The protons observed at δ=3.30 ppm were found to belong to *N*-methyl group. Thus, it turns out that the signals at δ= 50.6 and 51.7 ppm in the ^{13}C NMR spectrum belonged to two methyl groups on nitrogen atom.



Spectrum 7a: Extended HMBC spectrum *N*-methylcoryximine.

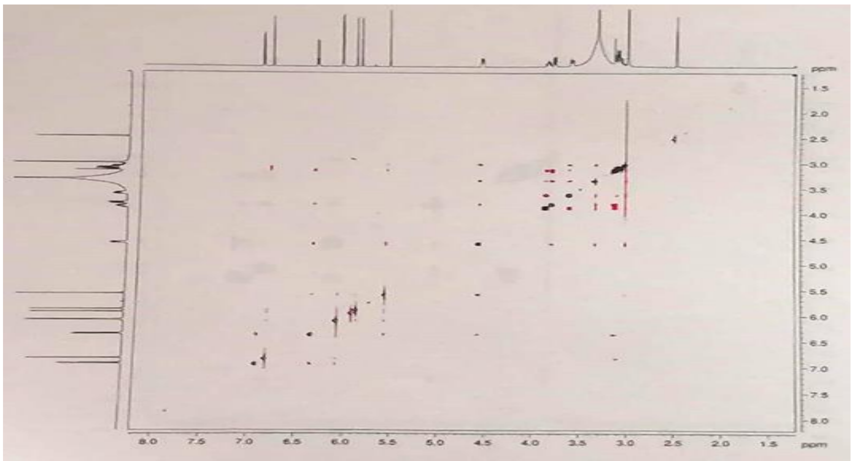
According to these correlations, the ring A and B is shown in Figure 2. Consequently, it has been proven that rings A and B form a quaternized isoquinoline structure with 6,7-methylenedioxy- *N*, *N*-dimethyl substituents, and with a substitution at the C-1 of this ring. In order to elucidate the substituent, the data obtained from the HMBC experiment was used.

In defining this aromatic structure, symbolized as the ring C, the $^4J_{CH}$ and $^3J_{CH}$ interactions of ortho-related hydrogens, which were at δ= 6.93 and 6.36 ppm, in the HMBC experiment were evaluated. One of the most interesting data during these evaluations is that the proton (H-6) resonating at δ=6.36 ppm interacts via three bonds with carbon at δ=34.7 ppm.



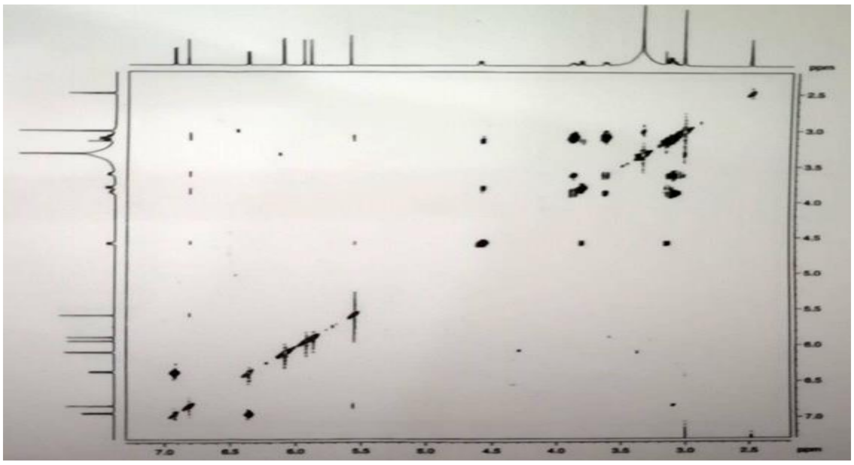
Spectrum 7b: Extended HMBC spectrum of *N*-methylcoryximine.

Another critical information about the structure was provided by the interaction of the proton signal (δ=6.36 ppm) with the carbonyl carbon signal (δ=165.9 ppm). Thus, the presence of a carbonyl residue on ring C emerged (Figure 3). The nature of the carbonyl group is evidenced by the broad signal of the proton of the carboxylic acid observed at δ=13.2 ppm in the ^{1}HNMR spectrum.



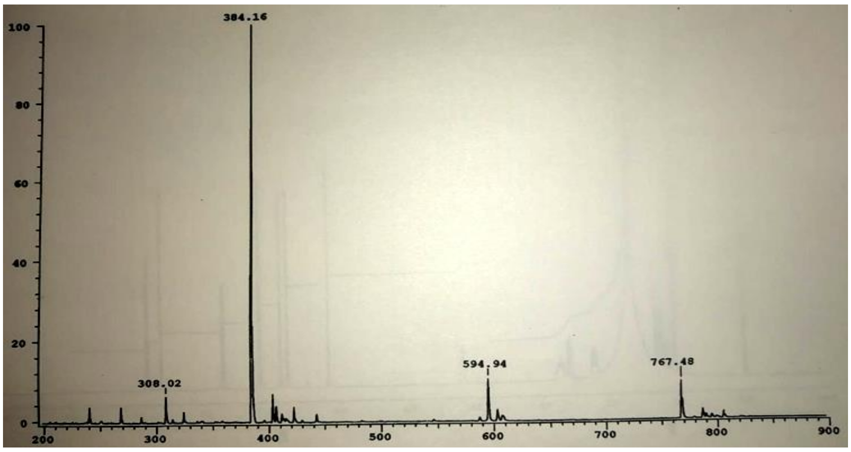
Spectrum 8: ROESY Spectrum *N*-methylcoryximine.

This finding was also consistent with the presence of the band for carbonyl group in the IR spectrum. All the above data with the molecular weight calculated as *m/z* 384 in ESI spectrum, verified the molecular formula of $C_{21}H_{22}NO_6$. A single *N*-methyl-bearing derivative of the non-quaternary compound known as coryximine. was previously isolated from a Papaveraceae plant called *Corydalis hsuchowensis*[17]. However, its total synthesis was also performed by Chrzanowska and Sulima in Poland in 1998[18]. Coryximine was also isolated from *Corydalis curviflora* and its larvicidal activity was demonstrated against two mosquito species[19]. A detailed literature search has shown that the quaternary derivative of coryximine, named as *N*-methylcoryximine, is a new compound that has not been registered in the literature until now. The levorotatory optical rotation of *N*-methylcoryximine in methanol indicated the *S* configuration at C-1 position. Although clear information was not provided from the ROESY experiment, it was thought that the ring C moved away from the *N,N*-dimethyl groups and approached to the ring A due to the steric density.



Spectrum 9: Tocsy spectrum of *N*-methylcoryximine.

This suggestion also explains the fact that H-8 had shifted to a very high field (δ=5.58 ppm). Therefore, it was concluded that the chemical structure of *N*-methyl coryximine was elucidated as depicted in Figure 4. The natural occurrence and spectral data of *N*-methylcoryximine from secoberbine subgroup of isoquinoline alkaloids, unprecedented to our knowledge, is reported for the first time. Further biological activity studies on this new compound are planned to be conducted.



Spectrum 10: ESI MS spectrum of *N*-methylcoryximine.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Dr. Tekant Gözler and Prof. Dr. Belkıs Gözler for their kind assisting in recording and interpreting NMR spectra. This project was funded by Ege University Scientific Research Council (98/ECZ/012).

CONFLICT OF INTEREST

Authors declare no conflict of interest associated with this work.

AUTHOR’S CONTRIBUTION

The design of the study and isolation process was carried out by EM. The interpretation of the data and drafting the document was done by HK and EM.

REFERENCES

1. Taborska E, Mikesova M, Veznik F, Slavik J. Alkaloids of the two *Hypecoum* L. species .Collect Czech Chem Commun 1987; 52:508-513. *https://doi.org/10.1135/cccc19870508*
2. Efros RS. Finding alkaloid-bearing folk-remedy plants in the Trans-Baikal. Region. Farmatsiya 1976; 9(5):22-23.
3. Sun B, Assani I, Wang C-G, Wang M-X, Liu L-F, Li Y, Yan G, Yang Y-R, Chen Z, Liao Z-X. Two new alkaloids from Tibetan medicine of *Hypecoum leptocarpum*, Nat Product Res 2021, 1-7. *https://doi.org/10.1080/14786419.2021.1937154*
4. Zhang GL, Rücker G, Breitmaier E, Mayer R. 1995. Alkaloids from *Hypecoum leptocarpum*. Phytochem 40(6): 1813–1816.
5. Zhou Y, Zhang G, Li B. Five alkaloids from *Hypecoum leptocarpum*. Phytochem 1999; 50:339-343.
6. Li BG, Zhou M, Zhang GL. Four new alkaloids from *Hypecoum leptocarpum* Hook. f. et Thoms. IJCB 2001; 40B (12):1215–1218.
7. Zhang Q, Luan G, Ma T, Hu N, Suo Y, Wang X, Ma X, Ding C. Application of chromatography technology in the separation of active alkaloids from *H. leptocarpum* and their inhibitory effect on fatty acid synthase. J Sep Sci 2015; 38:4063-4070. *https://doi.org/ 10.1002/jssc.201500848*
8. Su Y, Li S, Li N, Chen L, Zhang J, Wang J. Seven alkaloids and their antibacterial activity from *Hypecoum erectum* L. J Medicinal Plant Res 2011; 5(22):5428-5432. *https://doi.org/10.5897/JMPR.9001127*
9. Song P, Xia J, Rezeng C, Tong L, Tang W. Traditional, complementary, and alternative medicine: Focusing on research into traditional Tibetan medicine in Chin. Bio Science Trends 2016; 10(3):163-170. *https://doi.org/110.5582/bst.2016.01105*
10. Zhang R, Zha S, Yin X, Bai R, Li Y, Tu P, Chai X. Phytochemical and pharmacological progress on Tibetan medicine Hypecoi Erecti Herba and plants of *Hypecoum L.* Chin. Tradit Herb. Drugs 2016; 47:1217-1224. *https://doi.org/10.7501/j.issn.0253-2670.2016.07.0*
11. Wen H, Jiang L, Zhang D, Yuan X, Dang J, Mei L, Shao Y, Tao Y. Anti-inflammatory activity of total alkaloids from *Hypecoum leptocarpum* hook. f. et Thoms. Phcog Mag 2018; 14:397-403. *https://doi.org/10.4103/pm.pm_139_17*
12. Tao H , Zuo L , Xu H,, Li C, Qiao G , Guo M, Lin X. Alkaloids as anticancer agents: A review of chinese patents in recent 5 years. Recent Pat Anticancer Drug Discov 2020; 15(1): 2-13. *https://doi.org/10.2174/1574892815666200131120618*
13. Davis PH, Cullen J, Coode MJ. (1962-1988). Flora of Turkey and The East Aegean Islands. Edinburgh University Press, 1, 236.
14. Dahl AE. Taxonomic and morphological studies in *Hypecoum sect*. *Hypecoum* (Papaveraceae). Plant Systematics and Evolution 1989; 163: 227-280.
15. Mete E, Gözler T. (+)-Oxoturkiyenıne: an isoquinoline-derived alkaloid from *Hypecoum pendulum*. J Nat Prod 1988; 51:272-274. *https://doi.org/10.1021/np50056a013*
16. Abubakar A, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci 2020; 12(1): 1-10. *https://doi.org/ 10.4103/jpbs.JPBS_175_19*
17. Zhou JY, Tong XJ, Lian WY, Fang QC. Chemical Study on the Alkaloids of *Corydalis hsuchowensis*. Planta Med 1991; 57(2): 156-158. *https://doi.org/10.1055/s-2006-960054*
18. Chrzanowska M, Sulima A. Total synthesis of coryximine, a secophthalide isoquinoline alkaloid. Pol J Chem 1998; 72 (9): 2202-2203.
19. Xia JK , LiuY, Zhou Y , Zhang JY, Li CH , Yin X, Tian XR, Zhang XY. Two novel alkaloids from *Corydalis curviflora* Maxim and their insecticidal activity. Pest Manag Sci 2020; 76(7): 2360-2367. *https://doi.org/10.1002/ps.5772*