SOCIETÀ CHIMICA ITALIANA
Divisione di Chimica Farmaceutica

INNOVATION FOR HEALTH

SOCIETÀ CHIMICA ITALIANA
Divisione di Spettrometria di Massa

8° MS-PhArmaday

Complex substances for Botanical Drugs, Herbal Medicinal Products, Medical Devices, Medical Foods

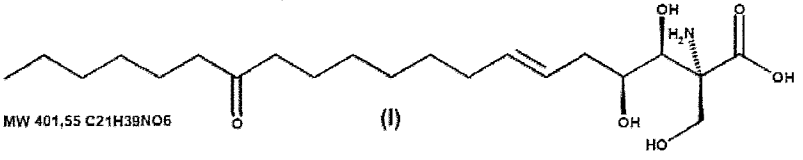
@ Aboca - Sansepolcro

June 11-13, 2014

CHARACTERIZATION OF MYRIOCIN AND SOME DERIVATIVES BY ELECTROSPRAY AND QTRAP MASS SPECTROMETRY

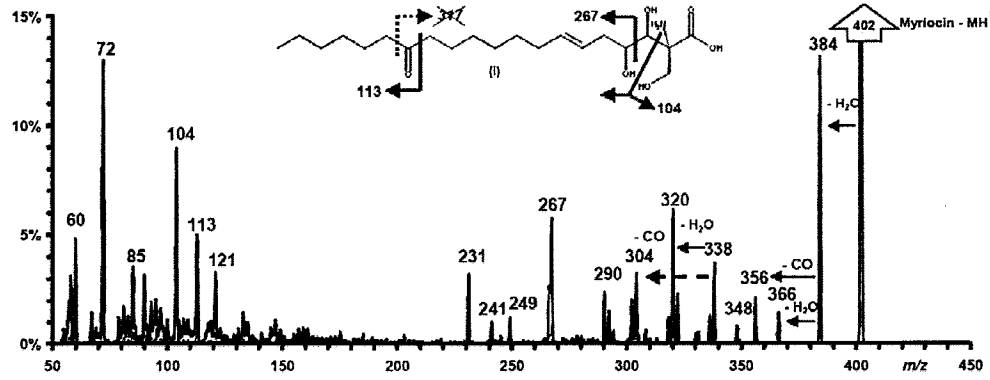
Federico Maria Rubino, Paola Signorelli, Jelena S. Lazarevic´, Rita Paroni*
Dipartimento di Scienze della Salute, Università degli Studi di Milano, v. A. di Rudinì 8, Milano (Italy)
*Department of Chemistry, Faculty of Medical Sciences, University of Nisˇ, Bul. dr Zorana Dindică 81, RS-18000 Nis (Serbia)

Myriocin [1] (or thermocydin, [2]) (**I**) was isolated and identified in the 1970s from fungi such as *Myriococcum albomyces*.



Mass spectrometry played at the time only a limited role in the structural elucidation due to the presence in the molecule of several high polarity functional groups: three hydroxyls, one primary amine, one carboxylic acid and one ketone carbonyl group. However, electron ionization of some derivatives yielded structurally diagnostic fragments, on the basis of which the mutual connectivity of the different functional groups in the 21-carbon chain could be assigned [3, 4]. Pharmacological studies on myriocin and on some synthetic analogues [5-9] later unveiled its strong pharmacological activity as an immunosuppressant and as an inhibitor of sphingosine synthesis and, very recently the mechanism for the inactivation of its target, serine palmitoyl transferase, was identified at the molecular level as involving the formation of a Schiff base with the cofactor pyridoxal phosphate [10]. Currently ongoing studies aimed at expanding its therapeutical use for the treatment of severe diseases such as retinitis pigmentosa [11] need a specific and sensitive analytical method based on mass spectrometry, since that recently published, which uses liquid chromatography with fluorescence detection of a derivative [12], may not be entirely adequate to the purpose. The characterization of underivatized myriocin with the new techniques of soft ionization and tandem mass spectrometry has not been so far carried and this limitation has hampered the setup of a suitable analytical method. We have thus studied the behaviour of myriocin and of some derivatives suitable for its analytical determination in biological samples by high-sensitivity mass spectrometry. All spectroscopic measurements were performed by infusing 1-10 microM solutions of the compounds in the ESI source of an API 3200Qtrap instrument and recording (collisionally activated) integrated fragment ion spectra of the protonated and deprotonated precursors in the profile mode over the range of nominal (laboratory)

collision energies from 5 to 50 V with the instrument's "*ramp CE*" feature in the Multi Channel Analysis mode. Fragment recording was accomplished either by scanning the Q3 or operating the Q3 in the ion trap mode. As an example, the spectrum of protonated (**I**) is reported. Unimolecular collisionally activated decomposition of protonated and of deprotonated myriocin performed in a triple quadrupole (Qtrap) instrument over a wide interval of collision energies (0,3-3.3 eV CoM) yields a poorly diagnostic fragmentation, mostly involving the sequential loss of small molecular units from the very polar and multiply functionalized head of the molecule and the threonine-connected head-group fragment, but little analytically useful fragmentation carrying the information on the position of the keto group, in particular for the deprotonated molecule. In the accessible domain of collision energies (0.3 to 3.2 e*VCM*) the multifunctional polar head of the molecules undergoes several expected fragmentation processes, both in protonated and in deprotonated molecular species, and yields low-mass fragments related to its threonine structural unit. Fragments representing the position and nature of the substituent along the hydrocarbon chain are of very low abundance and, as expected, only occur starting from relatively high collision energy (data not reported).



This unfavourable characteristics prompted to extend the study to some simple derivatives of the two most accessible functional groups in the molecule, also aimed at identifying the most useful strategy for labelling the molecule with stable isotopes to prepare an analytical internal standard with suitable characteristics for the very high sensitivity assay to be established. The analyzed derivatives of myriocin include: the (racemic) secondary alcohol deriving from reduction of the C-15 keto group; some O-substituted oxime derivatives of the C-15 keto group and Schiff bases of the C-2 amino group, which latter are also mechanistically important for the pharmacological mechanism of action of myriocin. The modification of the 15-C carbonyl by conversion into a secondary alcohol (**II**) seems not to modify fragmentation in the neighbouring of the keto-group. The oxime derivatives of the 15-C carbonyl group (**III**, **IV**) show

distinctive decomposition pathways in both polarities. In particular, loss of the *O*-substituent from the protonated oxime(s) yields a weak yet distinctive fragmentation product with the connectivity of a C-15 nitrile. The still undergoing study of the reaction of myriocin with aldehydes such as pyridine-3- carbaldehyde (Harvey's aldehyde) and pentafluoro-benzaldehyde shows both the formation of the cyclic acetals (mostly that of the 3,4-dihydroxy moiety) and of the Schiff base. Each class of aldehyde derivative can be discriminated by appearance of specific fragment ions.

1. Kluepfel, D., et al., J Antibiot (Tokyo), 1972. **25**(2): p. 109-15.
2. Craveri, R., P.L. Manachini, and F. Aragozzini, Experientia, 1972. **28**(7): p. 867-8.
3. Aragozzini, F., et al., Experientia, 1972. **28**(8): p. 881-2.
4. St-Jacques, M., J Org Chem, 1973. **38**(7): p. 1253-60.
5. Fujita, T., et al., J Antibiot (Tokyo), 1994. **47**(2): p. 216-24.
6. Yoshikawa, M., et al., Chem Pharm Bull (Tokyo), 1994. **42**(4): p. 994-6.
7. Yoshikawa, M., et al., Chem Pharm Bull (Tokyo), 1994. **42**(12): p. 2662-4.
8. Yoshikawa, M., et al., Chem Pharm Bull (Tokyo), 1995. **43**(10): p. 1647-53.
9. Oishi, T., K. Ando, and N. Chida, Chem Commun (Camb), 2001(19): p. 1932-3.
10. Wadsworth, J.M., et al., J Am Chem Soc, 2013. **135**(38): p. 14276-85.
11. Strettoi, E., et al., *Proc Natl Acad Sci U S A*. Oct 26, 2010; 107(43): 18706–18711.
12. Yu J, Xu H, Mo Z, Zhu H, Mao X. *Anal Sci*. 2009 Jul;25(7):855-9.