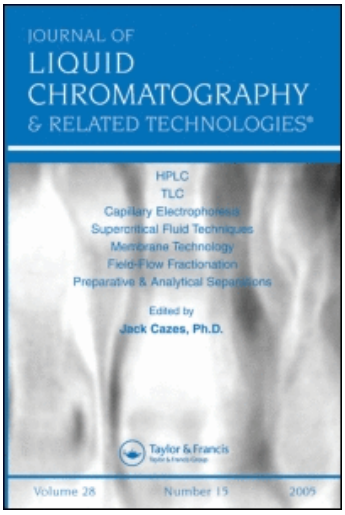

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Constituents of Higher Fungi. Part XVI. Identification of Lactarius Species by HPLC Using Sesquiterpene Monohydroxylactone Contents as Characteristic Chemotaxonomic Features

W. M. Daniewski[a]; W. Kroszczyński[a]; A. Wawrzuń[a]; A. Rymkiewicz[b]
[a] Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland [b] Institute of Biology and Pharmacy, School of Medicine, Wrarsaw, Poland

To cite this Article Daniewski, W. M. , Kroszczyński, W. , Wawrzuń, A. and Rymkiewicz, A.(1984) 'Constituents of Higher Fungi. Part XVI. Identification of Lactarius Species by HPLC Using Sesquiterpene Monohydroxylactone Contents as Characteristic Chemotaxonomic Features', Journal of Liquid Chromatography & Related Technologies, 7: 14, 2915 — 2920

To link to this Article: DOI: 10.1080/01483918408067056

URL: http://dx.doi.org/10.1080/01483918408067056

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(14), 2915-2920 (1984)

CONSTITUENTS OF HIGHER FUNGI. PART XVI.* IDENTIFICATION OF LACTARIUS SPECIES BY HPLC USING SESQUITERPENE MONOHYDROXYLACTONE CONTENTS AS CHARACTERISTIC CHEMOTAXONOMIC FEATURES.

W. M. Daniewski,[a] W. Kroszczyński,[a] A. Wawrzuń[a] and A. Rymkiewicz.[b]

[a]Institute of Organic Chemistry, Polish Academy of Sciences, 00-961 Warsaw, Poland.

[b]Institute of Biology and Pharmacy, School of Medicine, 51601 Wrocław, Poland.

ABSTRACT

It has been shown that the sesquiterpene monohydroxylactone contents of several species of mushrooms of the Lactarius family are characteristic chemotaxonomic features of the species. The profile of such compounds can easily be determined by HPLC and the resulting chromatograms can serve for purposes of identification of the species.

INTRODUCTION

In our previous investigations on mushrooms (1-3) of the Lactarius family, we isolated and identified several monohydroxylactones with lactarane and secolactarane skeletons. During our investigations (1), it was found that the monohydroxylactone content in each species was a characteristic chemotaxonomic feature. The contents could be determined by HPLC using a simple procedure and the chromatograms could serve for identification of these species.

*Part XV, Daniewski, W. M. et al, Tetrahedron, in press

 0148-3919/84/0714-2915$3.50/0

Identification of mushrooms is sometimes very difficult. In 1972, Professor A. Nespiak in Poland suggested to us the use of standardised TLC chromatograms of mushroom extracts for their identification. The TLC method is not accurate as compared with HPLC. In our previous paper (1) we published HPLC chromatograms of the monohydroxylactone contents of the following mushrooms: Lactarius necator, L. quietus, L. helvus, L. torminosus, L. glyciosmus and L. subdulcis. Now we wish to report the chromatograms of the following mushrooms: L. necator (standard), L. turpis, L. controversus, L. vellereus, L. pergamenus, L. vietus L. spinosulus and L. blennius.

EXPERIMENTAL

Materials

All the mushrooms were collected in Poland. Solvents were purified by standard procedures which included distillation just before use.

Apparatus

HPLC chromatograms were obtained with a home assembled isocratic liquid chromatograph, which included Waters 6000 SDS, U6K universal injector, Varian RI detector, and Chemipan IC-01 integrator. The column had 4 mm i.d., 300 mm length and was packed with 10μ Si60 Lichrosorb using a high pressure slurry technique (9000 theoretical plates).

Preparation of Extracts

Fresh mushrooms (30 - 50 g) were ground with ethanol (200 ml) and stored for 24 hours at ambient temperature. The slurries of

mushrooms were filtered through a sintered glass funnel covered with a 1 cm layer of cellite filter aid. The ethanol extracts were evaporated to dryness and dissolved in a 1:1 water/ether mixture (100 ml). The water layer was extracted with ether (3 x 75 ml) and the extracts were combined and the ether evaporated. The residue was prepurified by passing through short columns filled with alumina (neutral) in a solute/adsorbent ratio 1:15. The columns were eluted with a benzene/ethanol (7:3) mixture until lactarorufin A (TLC, green spot, H_2SO_4) appeared in the eluate. The evaporated eluates were rechromatographed on TLC plates (Merck, benzene/acetone 8:2). The zones of spots containing monohydroxylactones (Rf, 0.3 - 0.4) were separated, extracted with ethyl acetate, the solvent evaporated and the residue weighed.

Analytical Procedure

Before the analysis, the column was conditioned by passing through 20 volumes of solvent (hexane/ethylacetate 85:15). The flow rate was 1 ml/min. Purified samples were injected (ca. 0.5 mg) through the Waters U6K injector. The duration of every analysis was ca. 90 min. Since the Lactarius necator is a very common mushroom in Poland, its extract was found to be very useful for standardisation of these chromatograms. The retention volume of each lactone was checked against the volumes obtained by injection of the mixture from Lactarius necator to which blennin A had been added [4]. Any doubts about the identity of a peak could be cleared up by collecting samples of the lactone and measuring its mass spectrum.

TABLE 1

Peak No / Mushroom					1	2	3	4		5				7
L.necator	%				33.2	7.3	15.8	28.2		7.2				14.4
	time				42.5	45.0	47.1	51.5		55.8				69.2
	k'				8.44	9.0	9.5	10.4		11.4				14.4
L.controversus	%				9.7	54.7	35.6							
	time													
	k'													
L.vellereus	%				6.6	9.9	45.6	15.8						22.1
	time													
	k'													
L.turpis	%					3.4	66.1	11.2						19.3
	time													
	k'													
L.pergamenus	%	9.9	6.0		20.5	18.2	29.7					15.6		
	time	37.8	41.4									62.1		
	k'	7.5	8.2									12.34		
L.spinosulus	%				4.7	11.2	15.9	4.2	42.3				21.8	
	time								54.2				68.1	
	k'								10.75				13.5	
L.vietus	%				3.2	11.4	9.9	22.7		14.9	18.9		19.1	
	time										59.9			
	k'										11.9			
L.blennius	%				15.33	33.49	10.61	6.60				28.30		5.67
	time													
	k'													

RESULTS AND DISCUSSION

In figure 1 we can see the chromatograms of the monohydroxylactone contents of various species. The chromatogram of L necator has already been presented and serves as a standard (1). Table 1 summarizes the data obtained from the chromatograms. It was necessary to use a high capacity factor (k') in order to achieve separation. It was found again that the monohydroxylactone contents in the mushrooms investigated are characteristic features of every species. Extracts of Lactarius pergamenus, L. spinosulus and L. vietus contained new compounds in addition to the known monohydroxylactones. The compounds will be isolated in larger quantities and their structures will be investigated.

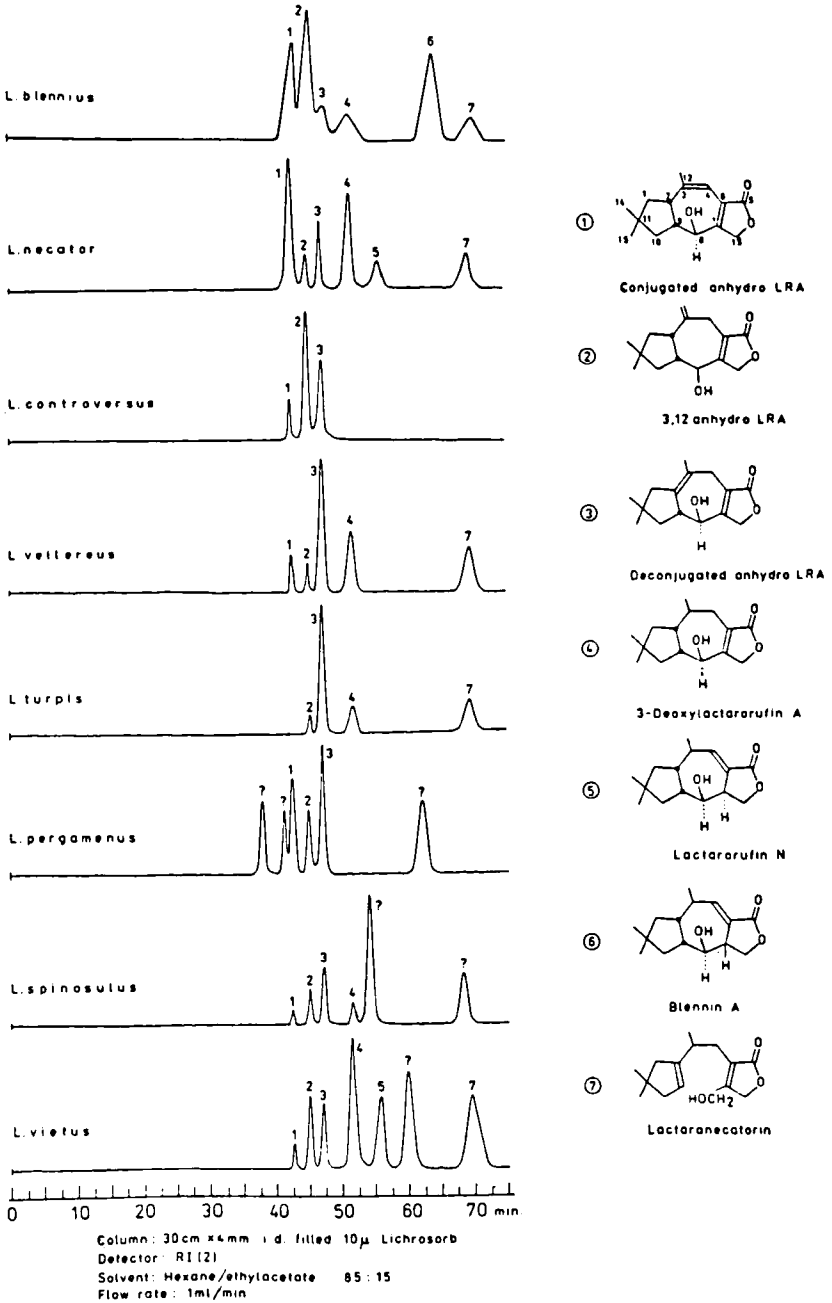

Figure 1. Chromatograms of the Monohydroxy-Lactone Contents of Various Species.

Lactarius controversus contained only three monohydroxylactones. Compound 3, the deconjugated anhydrolactarorufin A, was present in every species; thus it must be a very important intermediate in the biogenesis of these compounds. There are 70 species of Lactarius mushrooms in Poland and every one of them according to their supply will be investigated.

Monohydroxylactones play an important role in the life of these species as they have been found to be strong insect deterrents.(5)

ACKNOWLEDGEMENTS

We are grateful to Prof. Paola Vita-Finzi of the University of Pavia, Italy for providing us with a sample of blennin A, Mr. W. Ignacak for technical assistance, and The Polish Academy of Sciences for financial support under grant MR.I. 12. 1.5.3.2.

REFERENCES

1. Daniewski, W. M., Kocór, M., Januszewski, T., and Rymkiewicz, A., Polish J. Chem., 55, 807 (1981).

2. Daniewski, W. M., Kocór, M., and Król, J., Bull. Acad. Polon. Sci., ser chim., 23, 637 (1975).

3. Daniewski, W. M., Kocór, M., and Król, J., Roczniki Chem., 50, 2095 (1976). Ibid., 51, 1395 (1977).

4. Vidari, G., De Bernardi, M., Vita-Finzi, P., and Fronza, G., Phytochemistry, 15, 1953 (1976).

5. Nawrot, J., Bloszyk, E., Grabarczyk, H., Drożdż, B., Daniewski, W. M., and Holub, M., Prace Naukowe Instytutu Ochrony Roślin, Poznań, Poland, in press.