Turk J Chem
28 (2004) , 223 – 226.

3-Hydroxy Fatty Acids from the Flowers of *Hypericum lysimachioides* var. *lysimachioides*

Hasan Çetin ÖZEN[1]*, Mehmet BAŞHAN[1], Zuhal TOKER[1], Cumali KESKİN[2]
[1] *Dicle University, Faculty of Arts and Sciences, Department of Biology, 21280 Diyarbakır-TURKEY*
[2] *Dicle University, Mardin Health College, 47100 Mardin-TURKEY*
e-mail: hasano@dicle.edu.tr

Received 18.07.2003

Fatty acid methyl esters in the leaves and flowers of *Hypericum lysimachioides* var. *lysimachioides* (*Guttiferae*) were analyzed by gas chromatography and gas chromatography-mass spectrometry.

The flowers of *H. lysimachioides* var. *lysimachioides* produced unusual 3-hydroxy fatty acids [3-hydroxy- tetradecanoic acid (3-OH-C14:0) and 3-hydroxy-octadecanoic acid (3-OH-C18:0)], along with other normal fatty acids.

Major components were linolenic and palmitic acids for both leaves and flowers.

Key Words: *Hypericum lysimachioides* var. *lysimachioides,* fatty acid composition, 3-hydroxy fatty acid derivatives.

Introduction

The genus *Hypericum* is represented in the Flora of Turkey by 77 species[1]. The *Hypericum* species known as "binbirdelikotu" or "kantaron" in Turkish are used for their wound healing, antigastritis and antiseptic effects[2,3]. Nowadays purified extracts of their aerial parts are used for their antidepressant activity[4]. Furthermore, the anticancer[5] and antimicrobial activities[6] of hypericin are under investigation.

Studies on fatty acid composition show that the genus *Hypericum* is a rich source of omega-3 fatty acids[7–11].

3-Hydroxy fatty acids, that are unusual plant components are another class of fatty acids obtained from *Hypericum perforatum* and *Hypericum retusum*[9]. These fatty acids are an important class of microbial lipids that are used extensively to aid characterization of microorganisms[12]. In general, the fatty acid profile of most bacteria ranges from n-C_{10} to n-C_{18}. The fatty acids in most Gram-positive bacteria are located in the cytoplasmic membrane, though some, like mycobacteria and related genera have long chain lipids, known as mycolic acids (3- or ß-hydroxy fatty acids), which are found in an outer membrane-like structure. Gram-negative bacteria possess fatty acids in the cytoplasmic membrane, though they can also be found in the

*Corresponding author

cell wall fraction, generally in the lipopolysaccharide[12−13]. In spite of the existence some works on chemical constituents[14−16] little is known about the fatty acid composition of Turkish *Hypericum* species[9−11].

The purpose of this work was to evaluate the fatty acids of leaves and flowers of *Hypericum lysimachioides* var. *lysimachioides* by means of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Experimental

Plant Materials: Leaves and flowers of *H. lysimachioides* Boiss. & Noe. var. *lysimachioides* were collected near Diyarbakır in June 2002. The plant was identified by Dr. A. Selçuk Ertekin (Dicle University). Voucher specimens (No. DUF-9250) have been deposited in the Herbarium of the Department of Biology, Faculty of Sciences, Dicle University, Diyarbakır (Turkey).

Isolation and transmethylation of fatty acids

The isolation and transmethylation of fatty acids were carried out by the method described by Garches and Mancha[17]. Fifty grams of fresh plant material was heated with a mixture containing methanol, heptane, tetrahydrofuran, 2,2-dimethoxypropane and H_2SO_4 (37:36:20:5:2 in vol.). At 80 °C simultaneous digestion and lipid transmethylation took place in a single phase.

After cooling at room temperature, the upper phase was collected for gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis.

GC and GC-MS conditions

The fatty acid methyl esters (FAMEs) were analyzed by GC using a Unicam 610 gas chromatograph equipped with an SP-2330 capillary column (30 M x 0.25 mm, 0.2 μm film thickness, Spelco), a flame ionization detector and a Unicam 4815 recording integrator. The separations were conducted with temperature programming from 180 °C to 200 °C at 5 °C /min, after an initial 2 min hold. FAMEs were identified by comparison of retention times with authentic standards (Sigma Chemical Co.). As no standard was available for 3-OH-C18:0, identification was confirmed by GC-MS.

GC-MS analyses were conducted with GC-MS equipment (HP 5890-E Series GC System) with mass selective detection. An Innowax column (30 M x 0.25 mm i.d., 0.25 μm film thickness) was used, and the temperature was programmed from 150 °C to 230 °C at 2 °C min with an initial hold of 4 min and a final hold of 36 min. The carrier gas was helium (1 mL/min) and the split ratio was 50:1. The injection port was held at 250 °C and the detector at 300 °C. The mass spectrometer was operated in electron impact ionization mode (70 eV). FAMEs were identified by comparison with the Wiley 275 and Nist 98 databank.

Results and Discussion

The GC analysis of leaves and flowers of *H. lysimachioides* var. *lysimachioides* allowed the identification of 7 FAMEs in the leaves and 9 FAMEs in the flowers. The major constituent of the leaves and flowers was C 18:3, at 49.15% and 46.82% , respectively, and the minor constituent was C 14:0 for both parts of the plant (Table).

Table. Percentages of fatty acid in leaves and flowers of *H. lysimachioides* var. *lysimachioides.*[*]

FAMEs	RT	Leaves %	Flowers %	MS fragmentation pattern
C14:0	9.61	1.18 (0.08)	0.80 (0.04)	
C16:0	12.53	37.40 (0.85)	15.30 (0.60)	
C16:1	13.13	1.23 (0.07)	0.84 (0.06)	
C18:0	15.31	2.43 (0.10)	9.74 (0.63)	
C18:1	15.81	4.51 (0.67)	5.13 (0.82)	
C18:2	16.76	3.82 (0.53)	6.91(0.63)	
C18:3	17.95	49.15 (1.07)	46.82 (1.12)	
C14:0-3-OH	22.60	-	8.55 (0.59)	($C_{15}H_{30}O_3$): 258[M^+], 241(4), 199(3), 166(2), 141(3), 123(3), 111(6), 103(100), 97(6), 83(6), 71(19), 57(16), 43(23)
C18:0-3-OH[**]	23.67	-	5.50 (0.42)	($C_{19}H_{38}O_3$): 314[M^+], 310(3), 278(7), 241(4), 222(4), 213(3), 194(3), 141(1), 123(3), 103(100), 97(6), 83(9), 71(16), 57(13), 43(16)
Total		99.72 %	99.59 %	

[*]average data of 5 analyses

[**]identified by GC-MS

: not detected

RT: Retention time

The chain lengths of FAMEs were between C_{14} and C_{18}in *H. lysimachioides* var. *lysimachioides.* In the GC spectrum, the proportions of unsaturated FAMEs were higher than those at saturated ones. These results clearly indicate that both leaves and flowers of *H. lysimachioides* var. *lysimachioides* have similar FAME compositions except for 3-OH fatty acids. These fatty acids were only found in the flowers.

The structures of 3-OH-C14:0 and one unknown fatty acid (3-OH-C18:0) were further confirmed by GC-MS. The mass fragmentation patterns of these 2 FAMEs are given in the Table.

The mass spectra of 3-OH fatty acids showed their base peak at m/z = 103, which is indicative of a characteristic cleavage alpha to the carbon with the hydroxyl group[18]. The peaks at 166 for 3-OH-14:0 and 222 for 3-OH-18:0 are characteristic of 3-OH fatty acids, indicating a cleavage alpha to the carbon with the hydroxyl and containing water loss in the alkyl part of the molecules.

The presence of 3-OH fatty acids is of importance since their occurrence in the plant kingdom is rare.

References

1. N.K.B. Robson, *Hypericum.* In: P.H. Davis (editor), "**Flora of Turkey and the East Aegean Islands"** Vol. 2, pp 355-401, University Press, Edinburgh, 1967.
2. T. Baytop, **Therapy with Medicinal Plants in Turkey**, pp 185, İstanbul University Press, İstanbul, 1984.
3. **Endemic and Medicinal Plants of The GAP Area, Environment Foundation of Turkey** Publ. No. 143. Ankara, 2001.
4. C. Stevenson and E. Edzard, **Drugs 11**, 125-132 (1999).
5. A.R. Kamuhabwa, P. Agostinis, M. D'Hallewin, A. Karsan and P. De Witte, **Anticancer Res. 20**, 2579-2584 (2000).
6. A. Sokmen, B.M. Jones and M. Erturk, **Phytother. Res. 13**, 355-357 (1999).

7. F.D. Gunstone and G.M. Taylor, **Phytochem**. **6**, 1297-1298 (1967).
8. M. Girzu, A.P. Carnat and J.L. Chabard, **Ol**. **Corps Gras Li**. **2**, 317-318 (1995).
9. C. Keskin, Total lipid contents and fatty acid composition of some *Hypericum* species. Master's Thesis, Dicle University, 2002.
10. H.Ç. Özen and M. Başhan, **Adv. Food Sci**., **24**, 173-174 (2002).
11. H.Ç. Özen and M. Başhan, **Turk. J. Chem**. **27**, 723-725 (2003).
12. S. Laitinen, J. Kangas, K. Husman and P. Susitaival, **Ann. Agric. Environ. Med**. **8,** 213-219 (2001).
13. B. Szponal and L. Larson, **Ann. Agric. Environ. Med**. **8**, 111-117 (2001).
14. A. Çakır, M.E. Duru, M. Harmandar, R. Criminna, S. Passannanti and F. Piozzi, **Flavour Fragr**. **J**. **12**, 285-287, (1997).
15. S. Erken, H. Malyer, F. Demirci, B. Demirci and K.H.C. Baser, **Chem. Nat. Compd**. **37**, 434-438 (2001).
16. A. Çakır, A. Mavi, A. Yıldırım, M.E. Duru, M. Harmandar and C. Kazaz, **J**. **Ethnopharm**. **87**, 73-83 (2003).
17. R. Garces and M. Mancha, **Anal. Biochem**. **211**, 139-143 (1993).
18. M.M. Keinanen, L.K. Korhonen, P.J. Martikainen, T.Vartiainen, I.T. Miettinen, M.J. Lehtola, K. Nenonen and H. Pajunen, **J. Chromatogr. B**. **783**, 443-451 (2003).