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Fatty acid amides from freshwater green alga *Rhizoclonium hieroglyphicum*

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Abstract

Freshwater green algae *Rhizoclonium hieroglyphicum* growing in the Ural Mountains were examined for their fatty acid amides using capillary gas chromatography–mass spectrometry (GC–MS). Eight fatty acid amides were identified by GC–MS. (*Z*)-9-octadecenamide was found to be the major component (2.26%). © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Fatty amides; Green algae; *Rhizoclonium hieroglyphicum*

1. Introduction

Fatty acid amides are widespread in nature (Hannun et al., 1996). They were incorporated into some lipid molecules such as ceramides, glyco-sphingolipids, *N*-acylated lipids, and bacterial lipoproteins (Bezuglov et al., 1998; Schmid et al., 1990). Fatty acid amides are lipid bioregulators formed from long chain saturated and unsaturated fatty acids via amidation by the corresponding amines. Oleamides, the fatty acid ethanolamides, bind to the cannabinoid receptors of the central nervous system or peripheral tissues and can be considered as endogenous ligands of these receptors (Devane et al., 1992; Basile et al., 1999). Their pharmacological properties were discussed by Bezuglov et al. (1998) and di Marzo (1998).

Recently, we reported the composition of hydrocarbons and fatty acids of *Rhizoclonium hieroglyphicum* and found some unusual fatty acids (Rozentsvet et al., 1999). The present work reports the results of a continued investigation of natural fatty acid amides using gas chromatography–mass spectrometry (GC–MS).

2. Results and discussion

The composition of fatty acid amides in the green alga *R. hieroglyphicum* examined by capillary GC–MS is shown in Table 1. Mass spectrometry indicates the characteristic fragmentation of aliphatic amides. Table 1 indicates the molecular ion peaks of the amides and the abundant fragments at *m*/*z* 44 and m/z 59 which correspond to the loss of [O═C═N^+H_2] and [H_2C═C–NH_2–OH^+]. Peak at *m*/*z* 59 is characteristic of all fatty primary amides (Budziklewicz et al., 1964). Peak at *m*/*z* 72 corresponds to [H_2C═CH–C–NH_2–OH^+] and is found for synthetic oleamide (Hanus et al., 1999).

The study of the roots and leaves of some species of plants has shown that essential oil contains various amides. The lipophilic extracts from root and leaf of *Glycosmis trichanthera* are characterized by distinct chemical profiles, containing sulphur-containing

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Table 1
Mass spectral features of fatty acid amides identified in green algae *R. hieroglyphicum*

Natural amides	Abundance (%)[a]	M.W.[b]	*m/z* (rel. int.) 70 eV	References
1. 3-Methyl-butanamide	3.07 (0.20)[c]	101	59 (100), 44 (32), 43 (20), 43 (20), 41 (18), 57 (12), 86 (11), 73 (4) 101 (2.5)	Present study
2. 4-Methyl-pentanamide	4.30 (0.28)	115	59 (100), 44 (36), 72 (36), 43 (21), 41 (16), 57 (16), 100 (10), 86 (8), 115 (1)	Present study
3. Adipamide	5.53 (0.37)	144	59 (100), 44 (68), 86 (68), 72 (41), 41 (15), 55 (29), 99 (25), 43 (25), 4 (16) 39 (16) 111 (7) 144 (0.5)	Moreau et al. (1993)
4. 4-Ethyl-5-methyl-heptanamide	7.37 (0.48)	171	59 (100), 72 (47), 114 (28), 43 (19), 55 (18), 41 (16), 57 (15), 29 (9) 100 (5) 142 (2.5) 171 (1)	Present study
5. Dodecanamide	7.99 (0.52)	199	59 (100), 72 (31), 28 (21), 43 (24), 41 (17), 85 (8), 128 (6), 156 (4) 199 (1)	Present study
6. Hexadecanamide	14.44 (0.94)	255	59 (100), 72 (44), 28 (39), 43 (21), 41 (14), 86 (8), 128 (8), 170 (2) 212 (3) 255 (3)	Kawasaki et al. (1998)
7. (*Z*)-9-octadecenamide	34.72 (2.26)	281	59 (100), 72 (55), 41 (47), 43 (40), 55 (40), 69 (19), 67 (15), 114 (6) 128 (6) 154 (4) 212 (1) 281 (6)	Dembitsky et al. (2000)
8. Octadecanamide	22.58 (1.47)	283	59 (100), 72 (39), 28 (32), 43 (21), 41 (12), 128 (12), 198 (6), 240 (3) 283 (4)	Kawasaki et al. (1998)

[a] Percentage of total fatty acid amide.
[b] M.W. is the molecular weight.
[c] Percentage of total volatile compounds.

amides (Vajrodaya et al., 1998). Cherinonaine, a novel *Annona dimeric* amide, has been isolated from *Annona cherimola* (Chen et al., 1998). Two novel amides clausamides were isolated from the leaves of *Clausena lansium* (Li et al., 1996). Other bioactive amides such as hydroxycinnamates were isolated from plants (Faulds and Williamson, 1999). Nearly 600 constituents belonging to different classes of bioactive compounds including amides were isolated from genus *Piper* (Parmar et al., 1997).

Fatty acid amides are also found in grasses and microalgae. Kawasaki et al. (1998) have isolated two natural fatty amides — hexadecanamide (1.1%) and octadecanamide (1.2%) from the shoots of marine grass *Zostera marina*. Octadecanamide and other fatty amides were isolated from cyanobacterium *Aphannizomenon flos-aquae* (Dembitsky et al., 2000). Malyngamides, derivatives of tetradecanoic acid, were isolated from cyanobacterium *Lyngbya majuscula* (Wu et al., 1997). The cyclopropyl fatty amide, grenadamide, was detected in *Lyngbya majuscula* (Sitachitta and Gerwick, 1998). Branched-chain fatty amide was isolated from epiphytic dinoflagellate *Coolia monotis* (Tanaka et al., 1998).

Thus, our studies have shown, that freshwater algae *R. hieroglyphicum* (which is considered to be a dominating species in freshwater reservoirs), contains natural fatty amides.

3. Experimental

R. hieroglyphicum (Ag.) Kuetz. (Cladophoraceae, Chlorophyta) was harvested in July 1997 in the Shulgan river estuary (Bashkertostan Nature Reserve Shulgan-Tash Cave, Ural mountains, Russia). Freshly collected algae were thoroughly cleansed of extraneous matter, homogenized, and lipid extracted as described recently (Rozentsvet et al., 1999).

Separation of fatty acid amides was carried out with a GC Hewlett-Packard 5890 (series II) (Palo Alto, CA), equipped with a 5971 B MSD mass selective detector (Dembitsky et al., 1999). Fatty amides were analyzed by GC using serially capillary column with different stationary phases RTX-1 column 30 m × 0.32 mm ID, film thickness l μm, (Restek) coupled with a second capillary column RTX-1701 30 m long, 0.32 mm × 0.25 μm film thickness (Restek, PA). GC (initial temperature 40°C) was programmed at 2°C/min up to 300°C (20 min). Injector temperature was kept at 80°C. Helium carrier gas flow rate was 25 ml/s. The MS detector was operated at 194°C. Scan range was from 20 to 650 mHz at 0.9 scan/s. Fatty amides were identified by mass spectral library search (NBS75, Wiley 138 and 275), followed by comparing of the MS data with synthetic amides.

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