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UNCOMMON 16:1 (n-5) ACID FROM *DICTYOTA DICHOTOMA* AND FATTY ACIDS OF SOME BROWN ALGAE OF DICTYOTACEAE

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Abstract—The detailed fatty acid composition of the brown algae, *Dictyota dichotoma*, *Dictyopteris divaricata* and *Dictyota cervicornis* are reported. About 35 fatty acids were identified, of which 16:0, 18:4 (n-3), 18:1(n-9), 14:0 and 20:4(n-6) predominated in each sample. A high content of an uncommon 16:1(n-5) and the occurrence of C_{22} polyunsaturated fatty acids in minute amounts are characteristic features of algae from the genus *Dictyota* and *Dictyopteris divaricata*. The structure of 16:1(n-5) was confirmed by GC and mass spectrometry of its derivatives. The distribution of fatty acids among the polar lipids of *Dictyota dichotoma* are reported. The 16:1(n-5) acid was identified in glycolipids and betaine lipid.

INTRODUCTION

Algae of the Dictyotaceae provide a rich source of diterpenes of different structural classes and they have received much attention [1, 2]. Several authors believe that different kinds of biological activity (antifungal, antibacterial, etc.) of the Dictyotaceae are connected with the presence of these compounds [3–5]. However, other substances, for example, fatty acids (FAs) are also responsible for antifungal and allelopathic activities of brown algae [6, 7]. Information about the FAs of algae of the genus *Dictyota* is contradictory and very limited. There are some papers dealing with the fatty acid composition of *D. dichotoma* [8–11], but the data about even the main FAs are in disagreement. For example, 18:4(n-3) was the predominant polyunsaturated fatty acid (PUFA) in *D. dichotoma*, collected from the coast of Australia [8], but another acid, 18:3(n-3), was the main component among FAs in the same species collected in the Black Sea [9]. *Dictyota dichotoma* from the latter place was richer in the content of 20:4(n-6) and 20:5(n-3) [9] as compared with that of the same species from other regions [8, 10]. Neither 20:4(n-6) nor 20:5(n-3) have been found in *D. dichotoma* from the Arabian Sea [11].

In 1979, Johns *et al.* [8] detected a high content of a quite uncommon 16:1(n-5) in lipids of *D. dichotoma* (8% of total FAs). Later, other researchers did not find the same acid in the same species of *Dictyota* and they did not indicate double bond positions for FAs [9–11]. The presence of unusual fatty acids and the ratio of fatty acid isomers are valuable for taxonomic studies of macrophytic algae.

In this paper, evidence for the double bond position in the uncommon acid, 16:1(n-5), is reported, because the presence of this acid in *Dictyota* species may have some chemotaxonomic value. The FA composition of *D. dichotoma* and another two algal species of the Dictyotaceae (*D. cervicornis* and *Dictyopteris divaricata*) have also been studied and distinguishing features of the FA composition typical of these algae have been revealed. This report is part of our continued investigation of marine macrophytic algal lipids [12–14].

RESULTS AND DISCUSSION

The GC analyses on Supelcowax 10 M of the fatty acid methyl esters (FAMEs) of the three algal species studied showed, among recognized peaks for fatty acids, the presence of one uncommon peak (ECL 16.44). It was concentrated in the zone of monounsaturated FAs during separation of fatty acid methyl esters by $AgNO_3$–silica gel TLC. Upon catalytic hydrogenation this unusual FAME was converted to a palmitic acid ester. This result confirmed the presence of a double bond in the acid. We isolated C_{16} monounsaturated FAMEs using reversed-phase TLC [15]. To establish the position of the double bond of the uncommon acid, we used GC and mass spectrometry of its pyrrolidides and found that the double bond was located in the Δ^{11} position (or n-5) of the carbon chain of a 16:1 acid. The content of 16:1(n-5) was unusually high in all the algal species examined here, *viz*, 6.4–8.5% of total FAs. Johns *et al.* [8] were the first to find a high level of this acid in *D. dichotoma*. However, later, the fatty acid composition of the same *Dictyota* species was examined several times, but 16:1(n-5) was never detected [9–11]. Only recently, a large quantity of an uncommon isomer of 16:1(n-5) was found in *D. ciliata*, *D. dichotoma* and *D. naevosa* from the Senegalese coast (5.8–14.2% of all FAs) [16].

The acid 16:1(n-5) had been found in some red, brown and green algal species [17, 18] and seagrasses [19, 20], but only in minute amounts (< 0.4% of total FAs). Recently, a higher content (9.5–17.6%) was reported in red algae of the Solieraceae [21, 22]. Thus, algae belonging to the *Dictyota* and *Dictyopteris* are unique among Phaeophyta in containing a high percentage of 16:1(n-5). It may be presumed that the presence of this acid in large amounts is a chemotaxonomic feature of algal species of these two genera of the Dictyotaceae family.

The fatty acid compositions of the three brown algae studied are reported in Table 1. About 35 FAs were identified, but only seven of them accounted for over 72% of total FAs, 16:0, 18:4(n-3), 18:1(n-9), 14:0, 16:1(n-5), 20:4(n-6) and 20:5(n-3). It is well known that brown algae typically contain high amounts of C_{18} and C_{20} PUFAs [23, 24]. The same peculiarity was also found in Dictyotaceae species in the present work; 18:4(n-3) predominated among PUFAs in all species and the highest content was detected in *D. dichotoma* (18.0% of total FAs). This algal species from the Sea of Japan was richest in 18:4(n-3) among all the *Dictyota* species studied so far. This acid, together with 18:3(n-6), amounted to 8.8% in *D. dichotoma* collected near Australia [8], but the same species from the Black Sea and Senegalese coast contained less 18:4 (3.2% and 2.8% of total FAs, respectively) [9, 16]. Five *Dictyota* species from the Arabian Sea, however, did not contain 18:4(n-3) [11].

Arachidonic acid exceeded the level of 20:5(n-3) in the two *Dictyota* species; *Dictyopteris divaricata* had equal percentages of 20:4(n-6) and 20:5(n-3). These results are similar to those obtained from three *Dictyota* species collected near the Senegalese coast [16] and *Dictyopteris polypodiodes* [25]. The literature data on the presence and content of C_{20} PUFAs in the Dictyotaceae are most contradictory. In two Dictyotaceae species from the Black Sea, 20:5(n-3) was predominant among C_{20} PUFAs but 20:3 was not found. The algae from this region had the highest level of C_{20} PUFAs among all investigated species of the Dictyotaceae: *D. dichotoma* had 19.5% of 20:5 and 4.8% of 20:4; *Dilophus fasciola* had 21.1 and 13.2%, respectively [9]. Five *Dictyota* species from the Arabian Sea, *D. dichotoma* among them, contained neither 20:5 nor 20:4, and only *D. indica* was unusually rich in 20:3 (15.1% of total FAs) [11]. These authors did not indicate double bond positions in the acids although the presence of large quantities of both 20:3(n-6) and 20:3(n-3) is very unusual for algae [26, 27].

The real reasons for these contradictions on fatty acid composition from marine algae are unknown, but some authors believe that it may be the result of regional differences [11] or growth conditions [9]. Indeed, it has been shown that environmental factors (light, water

Table 1. Fatty acid composition of three brown algae (% of total FA content)

Fatty acid	*D. dichotoma*	*D. cervicornis*	*D. divaricata*
14:0	7.3	7.6	12.6
15:0	0.4	1.0	0.8
16:0	20.6	21.7	24.2
16:1(n-7)	0.9	1.2	2.3
16:1(n-5)	8.5	8.4	6.4
trans-16:1(n-13)	0.5	0.8	0.5
18:0	1.0	1.5	1.3
18:1(n-9)	10.7	15.2	10.8
18:1(n-7)	0.4	0.7	0.6
18:1(n-5)	0.7	tr	0.3
18:2(n-6)	2.4	2.8	3.1
18:3(n-6)	0.9	2.0	0.7
18:3(n-3)	3.8	2.6	5.4
18:4(n-3)	18.0	10.2	11.3
20:2(n-6)	0.4	3.2	0.5
20:3(n-6)	0.9	1.7	0.8
20:4(n-6)	9.5	7.5	4.1
20:4(n-3)	1.3	1.3	1.0
20:5(n-3)	5.8	5.0	4.1
22:4(n-6)	1.9	1.5	1.8
22:5(n-3)	0.4	0.2	0.4
22:6(n-3)	tr	tr	0.8
Other*	3.0	3.4	6.0
PUFAs (n-6)	16.0	18.7	11.0
PUFAs (n-3)	29.3	19.3	22.9

*Other: Minor acids (less than 0.5%): 12:0, *i* – 15:0, *i* – 16:0, 16:1 (n-9), 16:2 (n-6), 17:0, 17:1, 18:2 (n-4), 20:3 (n-3), 22:4 (n-3).

tr: Traces.

temperature, concentration of nitrogen and other compounds in water, etc.) influence the fatty acid content in algae [27, 28]. However, one may only presume that ecological factors alone are responsible for the considerable discrepancies of data on FAs of *Dictyota*, collected in different regions. Further work is required to determine whether environmental factors are able to change the ratio of FAs, e.g. 20:4(n-6), 20:5(n-3) and 20:3, in brown algae.

Several C_{22} PUFAs, 22:4(n-6), 22:4(n-3), 22:5(n-3) and 22:6(n-3), were usual components in the Dictyotaceae species examined here and 22:4(n-6) was predominant among them. Previously, only 22:4(n-6) and 22:5(n-3) had been found in *D. dichotoma* from Australian waters [8] and 22:4(n-6) and 22:3(n-6) for three *Dictyota* species from the Senegalese coast [16], whereas other reports did not contain any data on C_{22} PUFAs in Dictyotaceae [9–11, 25].

Dictyota dichotoma from the Sea of Japan and *D. cervicronis* from the South China Sea had similar FA profiles with some differences in the main acid contents. Hence, the site of collection did not affect the occurrence of C_{22} PUFAs and 16:1(n-5) in *Dictyota* species. So it may be presumed that the presence of these acids is a characteristic feature of the *Dictyota* and *Dictyopteris* species and does not depend on geographic region.

Investigation of the distribution of fatty acids in individual lipids of *D. dichotoma* (Table 2) showed that the 16:1(n-5) acid was located in glycolipids and betaine lipid. Its highest content was detected in sulphoquinovosyldiacylglycerol (SQDG) (11.5%). Phosphatidylglycerol (PG), the major phospholipid in *D. dichotoma* [29], contained *trans*-16:1(n-13) and traces of 16:1(n-7). In general, our results agree with those of Japanese workers who studied the distribution of FAs in individual lipids of *D. dichotoma*, but they did not indicate the double bond position of monounsaturated FAs [10]. Presumably, 16:1 in their study was mainly 16:1(n-5). The distribution of FAs in individual glycolipids of *D. dichotoma* was similar to that in other species of brown algae [10, 27, 30]. Monogalactosyldiacylglycerol (MGDG) was the most unsaturated lipid having the highest content of (n-3) PUFAs, mainly 18:4(n-3). Digalactosyldiacylglycerol (DGDG) was rich in 20:5(n-3) and had the same amount of 18:4(n-3). The most saturated lipid was SQDG having high proportions of 14:0 and 16:0. The betaine lipid, diacylglycerolhydroxymethyltrimethyl-β-alanine (DGTA) is distinguished from other lipids by a higher content of (n-6) PUFAs, mainly 20:4 and 18:2, 20:2, 20:3 and 22:4. These acids are chain links for the successive biosynthesis of PUFAs from 18:2 to 22:4 via elongation and desaturation.

Table 2. Fatty acid composition of lipid classes from *Dictyota dichotoma* (% of total FAs)

Fatty acid	MGDG	DGDG	SQDG	PG	DGTA
14:0	6.3	4.7	13.5	4.1	11.5
15:0	0.3	0.5	0.4	1.2	0.6
16:0	7.6	24.9	40.8	22.5	12.6
16:1(n-7)	0.4	0.5	tr	0.3	0.4
16:1(n-5)	9.4	9.6	11.5	—	7.7
16:1-*trans*	—	—	—	21.4	—
18:0	0.8	3.1	2.2	7.4	2.0
18:1(n-9)	9.6	4.0	13.4	7.3	12.1
18:1(n-7)	—	—	—	4.2	—
18:1(n-5)	0.4	0.6	1.0	0.3	0.9
18:2(n-6)	1.8	1.3	2.0	2.3	3.9
18:3(n-6)	1.1	0.7	0.1	0.1	0.1
18:3(n-3)	4.5	4.7	3.4	1.4	1.1
18:4(n-3)	41.6	17.2	1.1	0.8	0.9
20:2(n-6)	0.1	0.1	0.1	0.8	1.9
20:3(n-6)	0.3	0.8	0.5	1.0	1.8
20:4(n-6)	2.8	6.8	1.6	7.0	23.0
20:4(n-3)	1.7	0.8	0.5	6.8	1.3
20:5(n-3)	6.7	15.4	1.4	1.9	2.3
22:4(n-6)	0.4	tr	0.1	0.9	7.6
22:6(n-3)	—	0.4	0.5	tr	—
Other*	3.2	3.8	5.7	5.2	8.3
PUFAs (n-3)	54.5	38.1	6.4	10.1	5.6
PUFAs (n-6)	6.7	9.8	4.6	12.7	38.3

*Other: 12:0, *i* – 15:0, *i* – 16:0, 16:1(n-9), 17:0, 16:2(n-6), 20:0, 20:3(n-3), 22:4(n-3), 22:5(n-3).

MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol; PG, phosphatidylglycerol; DGTA, diacylglycerolhydroxymethyltrimethyl-β-alanine.

Thus, the results show that the presence of 16:1(n-5) in large amounts is the most important chemotaxonomic feature of the fatty acid composition of *Dictyota* and *Dictyopteris* species. The relatively high abundance of 18:4(n-3), 18:1(n-9), 14:0, 20:4(n-6), 20:5(n-3) and the occurrence of C_{22} PUFAs are characteristic features for these species of Dictyotaceae.

EXPERIMENTAL

Dictyota dichotoma and *Dictyopteris divaricata* were harvested in July and August 1991 and 1992 at 1–5 m depth in Peter the Great Bay of the Sea of Japan. *Dictyota cervicornis* was taken from the South China Sea near South Vietnam in March. Freshly collected algae were thoroughly cleaned from epiphytes, small invertebrates and sand particles.

Lipid extraction and fatty acid analyses. Combined samples from five to eight algal thalli of the same species were used for prepn of lipid extracts. Three to six samples of each algal species were analysed. Lipids were extracted by homogenization with $CHCl_3$–MeOH (1:2) [31]. The residue was re-extracted ×2–5 with small portions of $CHCl_3$–MeOH (1:1). Not less than two extracts of each sample were prepd and analysed. Fatty acids were converted to Me esters using 1% Na in MeOH, followed by 5% HCl in MeOH [32] and purified by silica gel TLC using C_6H_6. The resulting FAMEs were analysed by FID-GC using fused silica capillary columns (30 m × 0.25 mm), coated with Supelcowax 10 and SPB-5; column temps were 210° and 220°, respectively. The carrier gas was He at 40 ml min^{-1}, split 1:30.

Identification of fatty acids. Individual peaks of FAMEs were identified by comparing R_t data with those for authentic standards and using equivalent chain length (ECL) measurements [33]. In addition, FAMEs were fractionated according to their degree of unsaturation be prep. $AgNO_3$–silica gel TLC with hexane–Et_2O–HOAc (94:4:3). Frs were removed from the plate and extracted with $CHCl_3$ to obtain esters which were analysed by GC. Hydrogenation of Me esters was carried out in MeOH and catalytic amounts of PtO_2 at room temp. For the location of double bonds, fatty acid pyrrolidides were prepd by heating the Me esters in pyrrolidine with catalytic amounts of HOAc at 100° for 30 min [34]. Pyrrolidides were extracted and purified by TLC with $CHCl_3$–Me_2CO (9:1) and analysed by GC and MS.

Analysis of fatty acid composition of individual lipids. Lipids were sepd into individual classes by 2D TLC on precoated silica gel plates, using $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (50:20:10:10:0.4) in the first direction and Me_2CO–C_6H_6–HOAc–H_2O (200:30:3:10) in the second direction [35]. To identify lipids, specific spray reagents and comparison R_f values with those of authentic compounds was used [29]. To analyse FAs, individual lipids were visualized with fuchsin reagent [36], lipid spots removed and treated with 1% Na in MeOH, followed by 5% HCl in MeOH. The resulting FAMEs were analysed by GC.

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