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STEROLS AND FATTY ACIDS COMPOSITION OF CHAROPHYTES IN RELATION TO SOME OTHER GREEN PLANTS

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Abstract

Sterols and fatty acids composition of some members of green algae were analysed as tools for chemotaxonomy. Nearly from the orders of green algae, at least a representative member was chosen as a basic model for the order. Some bryophytan members were also studied for convenience. Owing to the debatable systematic position of *Vaucheria* it was also chosen and analysed together with the members of Chlorophyta.

The data obtained suggested an obvious relation between members of *Chara* and chlorophytes rather than between members of *Chara* and bryophytes. Members of *Chara* might be therefore considered to be green algae under a separate order "Charales", at the top of the orders in the green algae of the division of Chlorophyta. The results stand strongly against the idea that members of charophytes must be elevated to the rank of class or phyta. The data proved also that the sequence of the top three orders in Chlorophyta must be arranged as follows: Conjugales, Siphonales & Charales. Finally *Vaucheria* was found to be distant from Chlorophyta.

Introduction

It is probably true that sterols may serve as a taxonomic tool in plant classification (Smith, 1976) and subsequently they will be mentioned in relation to particular taxonomic situations (Goad & Goadwin, 1972; Grunwald, 1980; Erickson, 1983; & Matsuo & Atsuhiko, 1991).

Mohammady (1993), analysed the sterols of some green algae growing along the Mediterranean Seashore of Alexandria and concluded that sterols could be used as a chemotaxonomic parameters for classification of green algae.

Dunaliella and *Eudorina* (order: Volvocales) have been analysed for sterols (Granwell *et al.*, 1990). It was found that species of *Dunaliella* were characterized by complex distribution of Δ^5 ,Δ^7, $\Delta^{5,7}$ sterols. While *Eudorina* contains both Δ^5 and Δ^7 sterols only.

Systematic variation of fatty acids has been also studied by Nicholas (1970), Jamieson & Reid (1972), Mohammady (1993) and Abd El-Salam (1997). It is evident that many species have very different fatty acid compositions which may have value for taxonomic purposes. In general, it seems that major fatty acids are continuously distributed to form a satisfactory basis for taxonomic conclusions and therefore they are of potential interest to the taxonomists. Variations in the concentration of individual fatty acid have often been reported from organism to organism. Consequently, the use of quantitative variations may reinforce taxonomic data (Abd El-Salam, 1997).

The polyunsaturated fatty acids are believed to be absent in Cyanophyta, free-living Prochlorophyta and higher plants (Wood, 1974). They some times occur in great

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quantities and characteristics in many of Chlorophyceae, Xanthophyceae, Rhodophyceae, Phaeophyceae, Crysophceae, Hepatophyceae and Bacillariophyceae. It is also clear that the lower land plants, Bryophyta and Pteridophyta, contain varying amounts of these acids (Sewion, 1992).

Stefanov *et al.* (1988) concluded that there are strong similarities in the nature of the fatty acids found within genera although the relative proportions vary somewhat. In fact, most of the taxonomic correlations of fatty acids variation are revealed only by applying statistics (Wolf and Kwolek, 1971). Therefore, it may be concluded that fatty acids variation, to a certain extent, could be considered as a tool for chemotaxonomy.

In the present work, the authors studied the debatable position of charophytes in the plant kingdom were studied. A biochemical chemotaxonomic techniques based mainly on sterols and fatty acids composition were conducted as a trial to shed light on the interrelationship of charophytes with other plant taxa belonging to Chlorophyta and Bryophyta.

Materials & Methods

The biological materials were *Dunaliella Salina* (Volvocales), *Enteromorpha intestinalis* (L.) Link (Ulvales), *Cladophora fract*, Kg. (Cladophorales), *Oedogonium sp.* (Oedogoniales), *Spirogyra plena* (W. & G.S. West) Czurda (Zygnematales), *Caulerpa racemosa* (Forskal) K. Agardh & *Codium dichotomum* (Hudson) S.F. Gray (Siphonales). The studied charophytean members were *Chara hydropitys* Reich, *Chara contraria* Kutzing, *Chara delicatula* (Agardh) A. Braun and *Chara Zeylanica* Willdenow. Some bryophytan members namely *Funaria hygrometrica* (L.) Sbith, *Funaria serrata* Brid and *Tortula muralis* Hedw were also studied for convenience, beside *Vaucheria hamata*. They were collected from different localities: Abu-Qir, Montazah, Burg El Arab, Mex, Sidi Besher, Moharram Bay, Mariut lake, Rosetta, Damanhour, and Kafer- El-Shikh. For the identification of these biological materials, they were compared with the herbarium specimens of late professor A.H. Nasr(late professor of Phycology, Faculty of Science, Alex. University) found in our laboratory.

Lipid extraction: According to the method of Abd El-salam (1997).
Extraction of unsaponifiable lipids: According to the method of Abd El-salam (1997).
Isolation of sterols: According to the method of Nadal (1971).

Purification and identification of sterols: The sterol fractions separated and analysed using GLC; SE-30 chromatographic column. The identification of these fractions was carried out by comparing their relative retention time with that of published ones (Thompson *et al.*, 1980). The unknown sterol peaks were identified by comparison of their relative retention time related to cholesterol with that of reported data (Thompson, *et al.*, 1980). Purification and identification of sterols were conducted according to the method followed by Mohammady (1993). Fatty acids were then identified by comparison of their retention times with those of standards.

Estimation of individual sterol and fatty acid: The peak area of each individual fraction was calculated and the relative concentration of each fraction was expressed in percentage (Abd El-salam, 1997).

Results & Discussion

Table (1) shows the distribution of sterol fractions and their concentrations among the investigated plant materials. There were 24 sterol fractions, 16 of which were identified. Most sterol fractions were of the unsaturated forms but campestanol & 24-methyl pollinastanol were also presented. Data obtained from most sterol chromatograms showed the presence of two dominantly sterol factions found nearly in all the studied organisms. One of the two has a relatively small retention time and identified as z-22-Dehydrocholesterol, the other has a high retention time and identified as cycloartenol. 5,22,24-cholesta-trien-3β-ol was only found in *Oedogonium sp.* but *Spirogyra plena* was the only investigated alga contained brassicasterol. However, 7-ergost-en-3β-ol and 14α-methyl-5α-chloesta-7,22-dien-3β-ol were only detected in *Vaucheria hamata* beside one unidentified sterol fraction. The rest of sterol individuals were distributed among all the investigated members as shown in Table (1).

The similarity matrix of the studied plant materials based on the distribution of their sterol fractions was tabulated in table (2). While the similarity dendrogram was illustrated in Fig. (1). From the data recorded in table (2) it is obvious that the maximum degree of similarity (72.7%) was observed between *Codium dichotomum and Chara delicatula,* followed by (66.6%) observed bewteen *Funaria hygrometrica & Funira serrata* as well as between the genera of *Dunaliella & Enteromorpha, Chara & Tortula, Spirogyra& Codium* and also between *Caulerpa & Codium.* On the other hand, similarity value was observed between different *Chara species* and between the two species of *Funaria.* Members of Charophyta showed resemblance only to one member out of three to those of bryophytes, while showed resemblance to four members out of seven to taxa of green algae.

It was stated by many authors that: the more advanced sterols commonly distributed in higher plants have a methyl or ethyl group at C-24 on the side chain (Sikorsha and Farkas, 1982; Brunengo *et al.*, 1987; Akihisa *et al.*, 1987). Most of the phylogenetic systems considered that the higher plants originated, in their phylogeny, from ancestors of green algae.

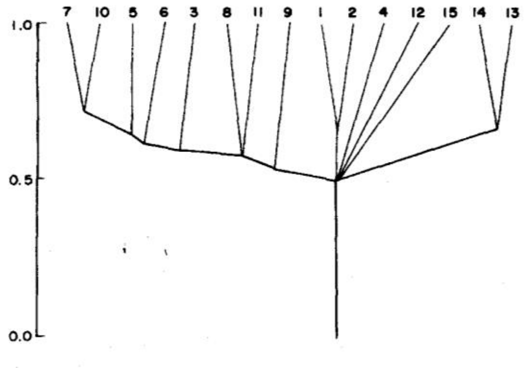

Fig. 1: Similarity dendrogram of the investigated members based on their distribution of sterols.

Table (1): Distribution of sterol fractions among the investigated members and their relative concentrations expressed as percentage.

Peak No.	Sterol	Investigated taxa														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
un.1	-	49.58	18.81			12.29	2.03	14.35						2.95	13.73	
un.2	-													5.91	27.47	
un.3	-	8.26	10.75	18.0	5.88	16.39		7.17	20.97	9.70	33.73					
1	Z-22-Dehydrocholesterol			12.0		12.29	12.19	11.48		11.47	13.88		3.19	9.46	8.24	
2	Cholesterol	11.57	9.67				12.19	14.35	6.29			18.78				14.36
3	5,22,24-cholesta-trien-3β-ol				13.07											
4	Brassicasterol					4.91										
5	14-α-Methyl-5α-cholest-7-en-3β-ol								4.19	11.76						
6	24-Methylenecholesterol					24.59						7.51				
7	14-α-Methyl-5α-cholesta-7,22-dien-3β-ol												4.25			
8	Campestanol								4.19	18.82				22.48		
9	Stigmasterol	11.36	17.20		11.76		35.77			15.29		13.14				
10	7-ergost-en-3β-ol												12.76			
11	24-Methylpollinastanol	3.71			11.76											
12	14α-Methyl-5α-ergast-8-en-3β-ol															
13	β-sitosterol	14.87								32.94	19.04				34.61	
14	5-α-stigmast-7-en-3β-ol						18.29	28.70	12.58		11.90	12.52				18.96
15	Cycloartenol			6.66	9.80	29.50		23.92	27.97		21.42	10.95		35.50		
16	24-Methylcycloartanol		24.19	50.0	29.41							8.89	53.19		8.24	3.44
un.4	-						19.51		27.97			28.17				54.59
un.5	-				18.30									23.66		8.62
un.6	-			13.33												
un.7	-															
un.8	-		19.35										12.27			

1. Dunaliella salina, 2. Enteromorpha intestinalis, 3. Cladophora fracta, 4. Oedogonium sp., 5. Spirogyra plena, 6. Caulerpa racemosa, 7. Codium dichotomum, 8. Chara hydropitys, 9. Chara contraria, 10. Chara delicatula, 11. Chara zeylanica, 12. Vaucheria hamata , 13. Funaria hygrometrica, 14. Funaria serrata, 15. Tortula muralis, Un. : Unknown

Table (2): The similarity matrix of the investigated members in accordance to their sterols distribution.

Operational taxonomic units	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	13%	14%	15%
1	-	66.6	36.3	50.0	16.6	33.3	33.3	30.7	50.0	36.3	30.7	18.1	16.6	16.6	36.3
2	-	-	36.6	46.1	33.3	50.0	50.0	44.4	33.3	18.1	46.1	18.1	16.6	16.6	36.3
3	-	-	-	50.0	54.5	18.1	63.3	33.3	36.3	60.0	33.3	40.0	18.1	36.3	40.0
4	-	-	-	-	30.7	18.1	30.7	28.5	16.6	36.3	28.5	20.0	30.7	15.3	33.3
5	-	-	-	-	-	16.6	66.6	30.7	33.3	54.5	30.7	18.1	50.0	50.0	18.1
6	-	-	-	-	-	-	66.6	46.1	46.1	36.3	46.1	18.1	33.3	50.0	36.3
7	-	-	-	-	-	-	-	61.5	46.1	72.7	30.7	18.1	50.0	50.0	36.3
8	-	-	-	-	-	-	-	-	46.1	50.0	57.1	0.0	30.7	33.3	50.0
9	-	-	-	-	-	-	-	-	-	54.5	15.3	18.1	33.3	16.6	18.1
10	-	-	-	-	-	-	-	-	-	-	33.3	20.0	36.3	36.3	40.0
11	-	-	-	-	-	-	-	-	-	-	-	16.6	15.3	30.7	66.6
12	-	-	-	-	-	-	-	-	-	-	-	-	18.1	18.1	20.0
13	-	-	-	-	-	-	-	-	-	-	-	-	-	66.6	18.1
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36.3
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. Dunaliella salina. 2. Enteromorpha intestinalis. 3. Cladophora fracta. 4. Oedogonium sp.. 5. Spirogyra plena. 6. Caulerpa racemosa. 7. Codium dichotomum. 8. Chara hydropitys. 9. Chara contraria. 10. Chara delicatula. 11. Chara zeylanica. 12. Vaucheria hamata . 13. Funaria hygrometrica. 14. Funaria serrata. 15. Tortula muralis.

The results for the distribution of sterol fractions revealed that nearly all of the methylated sterol fractions at C-24 were recorded in both green algae and stoneworts; while at the same time they were rarely found in the bryophytes. These results support the view that charophytes are more related to chlorophytes.

Similarity matrix and similarity dendrogram based on distribution of the same criterion indicate that *Chara hydropitys* and *Chara delicatula* are most related to *Codium dichotomum* (61.5% and 72.7% respectively followed by (60.0%) between *Cladophora fracta* and *Chara delicatula*. Concerning the similarity matrix and similarity dendrograms between members of charophytes and bryophytes, the maximum values of similarity (66.6% and 50%) were recorded between each of *Chara zeylanica* and *Chara hydropitys and Tortula muralis* (Table 2).

From a comparative point of view, and based on sterols composition, β-sitosterol (Stigmast-5-en-3β-ol; (24R)-24-ethylcholest-5-en-3β-ol) represents the most widely distributed sterol in higher plant (Sikorsha and Farkas, 1982; Brunengo *et al.*, 1987 and Akihisa *et al.*, 1987). Chlorophyta (Mohammady, 1993) and some members of Bryophyta (Marsili and Morelli, 1970). In our results this sterol was extracted from *Dunaliella salina*, two members of *Charales* and *Tortula muralis* and not extracted from other chlorophytan members. The results of the present work revealed that the studied taxa belonging to Charales have different advanced sterol fractions and accordingly reinforced the idea that Charales must be placed at the top of orders of green algae. Stigmast-7-enol (5α-stigmast-7-en-3β-ol) which was detected in *Acacia sp.* by Abd El-Salam (1997) and in both algae and insects (Thompson *et al.*, 1980) was observed in this work in *Codium dichotomum, Caulerpa racemosa, Chara hydropitys, Chara delicatula, Chara zeylanice* and *Tortula muralis*. Also a saturated nucleus fraction (Δ^{o}) campestanol (24R) 24-methyl-5 α-Cholestan-3β-ol); which is an advanced stanol; in our results it was found in both *Chara hydropitys* and *Chara contraria*. Taking into consideration the variations in sterols distribution, it was found that similarity matrix and similarity dendrogram as well as the stereogram agree with those of protein and isozymes (previously analysed by Abd El-Salam, 1997) that members of Charales are more related to members of Siphonales. The highest similarity value was (72.7%) this value can be observed between *Codium dichotomum* and *Chara delicatula*. The similarity value between *spirogyra plena* (*Conjugales*) and members of *Charales* is (54.5%) much lower than that between *Siphonales* and *Charales* (72.7%). At the same time, the taxonomic stereogram concerning sterols distribution revealed the same conclusion. Two members out of four of charophytes showed resemblance to Siphonales (50% resemblance), while only member out of four showed resemblance to Conjugales (25% resemblance). At the same time, these results are against those of Stewart and Mattox (1975) they considered that Cytologically Conjugales are more related to Charales than Siphonales. This forced them to consider Conjugales more advanced than Siphonales.

The obtained GLC data concerning fatty acids distribution (Table 3) gave fatty acid fractions with varied carbon chain lengths ranged from C 8:0 to C 24:0 with differences in the degree of saturation. Eleven saturated fatty acids and seven unsaturated ones were detected. The distribution of fatty acid fractions and their relative concentration differed according to the studied genera. C 10:0 demonstrates the mostly wide distributed fraction between the OUT'S, while C 20:1 shows a minimum representation of these fractions. However, the green algae contained nearly most of these fatty acids (77.7%)

followed by charophytes (61.1%) then bryophytes (50.0%), the distribution, however shows more variation between the bryophytan taxa.

The similarity matrix of the studied plant materials based on their fatty acids distribution was tabulated in Table (4). While the similarity dendrogram and taxonomic stereogram of these plant materials were shown in Figures (2 & 3). The data reflected considerable relation between the investigated members. The highest similarity value (92.8%) was found between *Oedogonium sp.* and *Spirogyra plena.* Other high similarity values were also recorded between *Dunaliella salina* and *Chara delicatula* (88.8%), *Enteromorpha intestinalis* and both *Oedogonlum sp.* and *Spirogyra plena* (86.6%), and

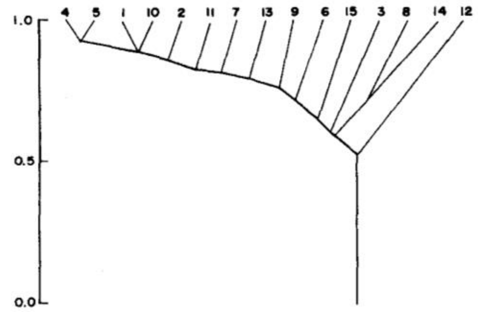

Fig. 2: Similarity dendrogram of the investigated members based on their distribution of fatty acids.

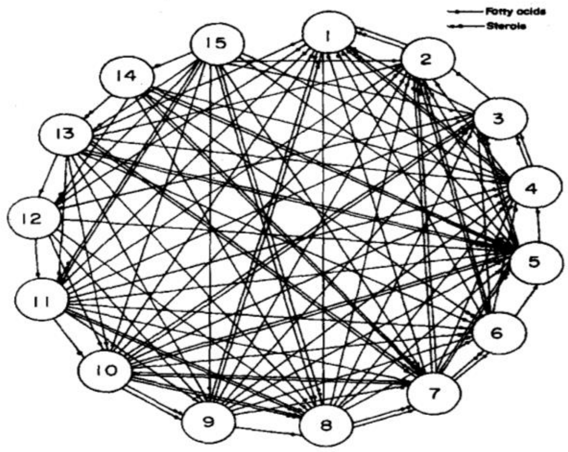

Fig. 3: Taxonomic stereogram showing similarity bonds (concerning sterols and fatty acids) between the investigated members. Similarities above 50% were considered.

1. *Dunaliella salina*	**6. *Caulerpa racemosa***	**11. *Chara zeylanica***
2. *Enteromorpha intestinalis*	**7. *Codium dichotomum***	**12. *Vaucheria hamata***
3. *Cladophora fracta*	**8. *Chara hydropitys***	**13. *Funaria hygrometrica***
4. *Oedogonium sp.*	**9. *Chara contraria***	**14. *Funaria serrata***
5. *Spirogyra plena*	**10. *Chara delicatula***	**15. *Tortula muralis***

Table (2): The similarity matrix of the investigated members in accordance to their sterols distribution.

Operational taxonomic units	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	13%	14%	15%
1	-	66.6	36.3	50.0	16.6	33.3	33.3	30.7	50.0	36.3	30.7	18.1	16.6	16.6	36.3
2	-	-	36.6	46.1	33.3	50.0	50.0	44.4	33.3	18.1	46.1	18.1	16.6	16.6	36.3
3	-	-	-	50.0	54.5	18.1	63.3	33.3	36.3	60.0	33.3	40.0	18.1	36.3	40.0
4	-	-	-	-	30.7	18.1	30.7	28.5	16.6	36.3	28.5	20.0	30.7	15.3	33.3
5	-	-	-	-	-	16.6	66.6	30.7	33.3	54.5	30.7	18.1	50.0	50.0	18.1
6	-	-	-	-	-	-	66.6	46.1	46.1	36.3	46.1	18.1	33.3	50.0	36.3
7	-	-	-	-	-	-	-	61.5	46.1	72.7	30.7	18.1	50.0	50.0	36.3
8	-	-	-	-	-	-	-	-	46.1	50.0	57.1	0.0	30.7	33.3	50.0
9	-	-	-	-	-	-	-	-	-	54.5	15.3	18.1	33.3	16.6	18.1
10	-	-	-	-	-	-	-	-	-	-	33.3	20.0	36.3	36.3	40.0
11	-	-	-	-	-	-	-	-	-	-	-	16.6	15.3	30.7	66.6
12	-	-	-	-	-	-	-	-	-	-	-	-	18.1	18.1	20.0
13	-	-	-	-	-	-	-	-	-	-	-	-	-	66.6	18.1
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36.3
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. Dunaliella salina, 2. Enteromorpha intestinalis, 3. Cladophora fracta, 4. Oedogonium sp., 5. Spirogyra plena, 6. Caulerpa racemosa, 7. Codium dichotomum, 8. Chara hydropitys, 9. Chara contraria, 10. Chara delicatula, 11. Chara zeylanica, 12. Vaucheria hamata , 13. Funaria hygrometrica, 14. Funaria serrata, 15. Tortula muralis.

Table (4): The similarity matrix of the investigated members based on their fatty acids distribution.

Operational taxonomic units	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%	11%	12%	13%	14%	15%
1	-	86.6	54.5	78.5	78.5	69.5	66.6	63.6	76.9	88.8	80.0	50.0	69.2	40.0	45.4
2	-	-	58.3	86.6	86.6	64.0	69.2	66.6	78.5	82.7	74.0	43.4	78.5	54.4	58.3
3	-	-	-	54.4	54.5	58.8	44.4	62.5	60.0	50.0	52.6	53.3	41.6	42.8	57.1
4	-	-	-	-	92.8	78.2	83.3	63.6	76.9	78.5	80.0	38.0	84.6	50.0	63.6
5	-	-	-	-	-	69.5	83.3	63.6	76.9	81.4	80.0	47.6	81.4	50.0	63.6
6	-	-	-	-	-	-	63.1	55.5	66.6	72.7	70.0	37.5	66.6	40.4	47.0
7	-	-	-	-	-	-	-	55.5	63.6	78.2	66.6	47.0	81.8	62.5	66.6
8	-	-	-	-	-	-	-	-	60.0	66.6	73.6	53.3	50.0	71.4	50.0
9	-	-	-	-	-	-	-	-	-	72.0	69.5	55.5	75.0	44.4	50.0
10	-	-	-	-	-	-	-	-	-	-	83.3	52.6	80.0	42.1	57.1
11	-	-	-	-	-	-	-	-	-	-	-	35.2	60.8	47.0	52.6
12	-	-	-	-	-	-	-	-	-	-	-	-	52.6	61.5	53.3
13	-	-	-	-	-	-	-	-	-	-	-	-	-	44.4	63.6
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	57.1
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. Dunaliella salina, 2. Enteromorpha intestinalis, 3. Cladophora fracta, 4. Oedogonium sp., 5. Spirogyra plena, 6. Caulerpa racemosa, 7. Codium dichotomum, 8. Chara hydropitys, 9. Chara contraria, 10. Chara delicatula, 11. Chara zeylanica, 12. Vaucheria hamata, 13. Funaria hygrometrica, 14. Funaria serrata, 15. Tortula muralis.

*** out of the range of our estimation**

also between *Dunaliella salina* and *Enteromorpha intestinalis* (86.6%), *Oedogonium sp.* and *Funaria hygrometrica* (84.6%), *Oedogonium sp.* and *Codium dichotomum* (83.3%), *Spirogyra plena* and *Codium dichotomum* (83.3%), as well as *Chara delicatula* and *Chara zeylanica* (83.3%), *Codium dichotomum* and *Funaria hygrometrica* (81.8%), and between *Spirogyra plena* and both species *Chara delicatula* and *Funaria hygrometrica* (81.4%). On the other hand, the lowest similarity value (35.2%) was recorded between *Chara zeylanica* and *Vaucheria hamata* followed by (38%) which was recorded between *Oedogonium sp.* and *Vaucheria hamata*. Generally, it is clear that most of the investigated members are tied together with a degree of similarity above 50%.

Although the stereogram concerning fatty acids distribution (Fig. 3) showed that members of both Chlorophyta and Bryophyta are equally tied to Charophyta (100% resemblance), yet the similarity values were different. The highest similarity value between members of charophytes and members of chlorophytes was (88.8%) while the highest similarity matrix between members of charophytes and those of bryophytes was (80.0%). At the same time, the individual dendrogram reveals also the same conclusion. It was found by Aknin *et al.* (1992) that C 16:1 fatty acid originates in chloroplasts and it is widely distributed in leaves of higher plants. Since the algal ancestors of the land flora was a filamentous terrestrial green alga like *Fritschiella* (McBride, 1970 and Floyd *et al.*, 1971) or *Terntepolly* (Graham and McBride, 1974), therefore the predominance of this unsaturated fatty acid (C 16:1) in the green algae and stoneworts in the present work and its absence from bryophytes may indicate that the first two plant groups are related to each other than the third one. It was believed that bryophytes have an evolutionary pattern of their own for fatty acid biosynthesis and cannot be described as an intermediate stage between lower and higher plants (Sewion, 1992). This idea was supported also by many authors (Stewart and Mattox, 1975) they concluded that Charales and Coleochaetales (order of green algae) are most related to each other cytologically. They also added that Charales and Coleochaetales are the most highly advanced algae cytologically similar to Archegoniatae plants. From the similarity matrix based on fatty acids distribution, there is a very close correspondence between all the studied members belonging to Charales or belonging to the other orders of green algae. Accordingly, it seems difficult to determine the relation of these orders to each other. However, from the data based on fatty acids distribution it is obvious that Oedogoniales and Conjugales have the highest similarity value (92.8%), a result which confedrate with the opinion that these two orders are generally agreed to have been derived long ago from common ancestors (Pickett-Heaps, 1975). The average similarity matrix and average dendrogram as well as the average taxonomic stereogram concerning fatty acids and sterols are on the same line with the results obtained previously by Abd El-Salam (1997) for protein and some isozymes that members of *Chara* are more related to members of Siphonales rather than to conjugales. The highest average similarity value could be recorded between *Codium dichotomum* and *Chara delicatula* (78.2%).

The above data concerning the sterols and fatty acids composition revealed that members of *Chara* are merely green algae. The results stand strongly against the idea that members of *Chara* must be elevated to the rank of class or phyta. There is a clear relation between members of *Chara* and chlorophytes rather than between members of *Chara* and bryophytes. Members of *Chara* must be placed as a separate order "*Charales*" at the top of the orders within Cholorophyta. The sequence of the top three orders in Cholorophyta must be arranged as *Conjugales*, *Siphonales* and *Charales*. However, *Vaucheria* was found to be distant from Chlorophyta.

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الاسترولات والأحماض الدهنية فى النباتات الكارية وبعض النباتات الخضراء الأخرى

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تهدف الدراسة الحالية إلي إلقاء الضوء علي العلاقات التصنيفية بين بعض أنواع جنس كارا وأخرى تنتمي لكل من شعبتي الطحالب الخضراء والنباتات الحزازية وذلك باستخدام الأسترولات والأحماض الدهنية كدليل من الأدلة المستخدمة في التصنيف كما استخدمت الطرق الإحصائية لتوضيح علاقات التشابه بين أفراد تلك النباتات علي أسس رياضية من خلال جداول تقييم التشابه وأشكال فراغية تظهر بوضوح هذه العلاقات التصنيفية من خلال علاقة الطحالب الكارية بغيرها من المجموعات النباتية الأخرى وترتيب رتب الطحالب الخضراء وكذلك الوضع التقسيمي لطحلب فوشيريا. وقد أوضحت النتائج أن الطحالب الكارية ليست إلا طحالب خضراء ترتبط بعلاقات أقوى مع الطحالب الخضراء عن ارتباطها مع النباتات الحزازية. كذلك وجد أن الطحالب الكارية وطحالب رتبة سيفونالس ترتبط بأعلى قيم تشابه وعليه فإن الطحالب الكارية تقع كرتبة على قمة رتب الطحالب الخضراء. كذلك تم ترتيب الرتب الثلاثة العليا داخل الطحالب الخضراء على النحو التال: رتبة كونجيوجاليس ثم رتبة سيفوناليس وأخيراً رتبة كاراليس. ومن النتائج أيضا يتضح تباعد طحلب فوشيريا لدرجة لا يمكن بها وضع هذا الطحلب ضمن الطحالب الخضراء.