# A new application of oxyreactive thermal analysis in marine algological studies

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Algae
Methodology
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Taxonomical differentiation

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**Abstract**

This is a preliminary study of the application of oxyreactive thermal analysis in algological investigations. Several species of Chlorophyta, Phaeophyta, Rhodophyta and *Zostera marina* taken from different stations off the southern Baltic coast have been studied. It is pointed out that oxyreactive thermal analysis can be used for taxonomical investigations in order to establish the systematic membership of certain species of algae based on fragments of thallus. This method can also be applied in order to establish environmental specificity by differentiating the chemical composition of certain species. It is also suitable for assessing biochemical differentiation among the various parts of the thallus.

## 1. Introduction

In the taxonomy of algae a number of criteria are applied as methodological tools in identification work. One of these tools involves determining the chemical composition of cells, and is defined in the literature as a biochemical one (Baraskov, 1972; Kadłubowska, 1975). As a result of numerous investigations, the chemical composition of several algae species is quite well known. It is generally understood that some chemical compounds are specific to a certain taxonomical group. A key to the identification of algal divisions based on the occurrence of certain pigments or substances stored in the cell has even been compiled. In many cases the presence of individual

chemical substances is specific to lower taxonomical units. These compounds can be stored in greater quantities in particular parts of the thallus, *e.g.* in brown algae, alginic acid occurs in the intercellular space and in the cell wall.

Thermal analysis (TA) is an analytical technique for determining the physico-chemical features of different substrata of organic origin and in recent years has been used more and more frequently in very different fields of research into organic compounds and substrata. In such investigations a version of TA is very often used in which heating takes place in a neutral atmosphere. In this case the phase transformation processes and the effects of the thermal dissociation of the compounds in question are investigated.

Another version of TA is performed in an oxidising atmosphere. However, apart from the above-mentioned processes, oxidation of the thermal dissociation products of the sample components or of the non-dissociating compounds takes place. Oxidation reactions are the principal reactions applied in classic chemical analysis; they play an important role in the analysis of carbohydrates, which make up from 40 to 80% of algal matter.

Owing to its insufficient methodological preparation, TA conducted in an oxidising atmosphere has been applied but infrequently in investigations of the composition and structure of organic compounds. However, a literature review of the oxyreactive TA in 1970–1980 (Wesołowski, 1981) refers to 53 articles on studies of different hydrocarbons. Several other works (Bihari-Varga, 1982; Kosik *et al.*, 1982; Wesołowski, 1985, 1986a,b, 1987; Anghern-Bettinazi *et al.*, 1988) have also pointed out that this method could be applied in the physicochemical analysis of biological matter. The results of the research done by one of us on a variety of organic substances recommended this version of TA for investigations into organic compounds (Cebulak and Szwed-Lorenz, 1990; Cebulak, 1992), especially in taxonomic studies (Ramos-Sanches *et al.*, 1991; Cebulak *et al.*, 1993a; Cebulak *et al.*, 1993b).

The main aim of this work was to verify the efficacy of oxyreactive TA for differentiating the chemical composition of algae. It was intended to determine to what extent the results of analyses could be used to identify the physicochemical specificity of algae, and the results were expected to confirm the practicability of this method in the taxonomic investigation of marine algae. The next step will be to discover the extent to which this method can be used in general investigations of algae.

Algae are a part of the plant kingdom displaying great diversity of composition: the evolutionary development of algae has been such that the most primitive forms exist alongside highly-developed ones. Assuming photosynthesis to be of equal efficacy in both terrestrial and aquatic plants, and

taking the productivity of the biomass in the various biotopes into consideration, it has been calculated that from 1/2 to 9/10 of the global quantity of organic matter and oxygen has been produced by algae (Kadłubowska, 1975). These characteristics of algae are another reason why this work has aimed to find whether TA could be applied in biological and ecological investigations.

The main chemical constituents of algae are carbohydrates, fats and proteins. The differences in the content of these constituents in various groups of algae are shown in Tab. 1.

**Table 1.** The chemical composition [%] and energy value (in calories) of 100 g algal organic matter (after Kadłubowska, 1975)

| Algal group | Proteins | Carbohydrates | Fats | Energy value |
|---|---|---|---|---|
| brown algae + red algae | 20 | 79 | 1 | 415 |
| green algae | 45 | 43 | 12 | 472 |
| blue-green algae | 30 | 64 | 6 | 441 |
| diatoms | 40 | 30 | 30 | 525 |

The compounds mentioned in Tab. 1 are the principal constituents. Colouring substances, like chlorophyll, xanthophyll and carotene, responsible for photosynthesis, are present in much smaller quantities. Furthermore, alkaloids – characteristic toxic substances – are present in certain divisions or classes of algae. More detailed research has revealed in algae a wide diversity of carbohydrates, from which both storage and cell body materials are built.

Some divisions, such as the Cryptophyta or Chlorophyta, produce starch as storage material, which is sometimes characteristically differentiated as cyanophytean starch (Cyanophyta), or floridean starch (Rhodophyta) – also known as agar-agar. Some divisions contain starch and lipids (Pyrrophyta), others produce chrysolaminarin (Chrysophyta) or paramylon (Euglenophyta and Xantophyceae), while laminarin and mannitol occur in brown algae (Phaeophyta). There is also one class in which the storage material consists of lipids (Raphadophyta).

The cell-wall composition is specific to certain divisions or classes. Cellulose and pectins are the most common constituents, but sometimes chitin (some Chrysophyta) or hemicellulose (some Chlorophyta) are found as well. Some divisions and classes are characterised by pectins containing large quantities of Ca or Si in their structure. There are other divisions in which the cell-walls consist of saccharin-protein compounds – *e.g.*

the so-called mureins in Cyanophyta and Prochlorophyta, or the strips of protein-lipid-carbohydrates in Euglenophyta.

All carbohydrates are polysaccharides with an intricate structure of extensively branched chains. This structure is further diversified by the addition of ions such as Ca, Si, and sometimes Na at various sites in the algin-compounds characteristic of certain groups of algae. Starch compounds have been found to display differences, due not only to the presence of straight or branched-chain forms, but also to the interrelationships between them. They are additionally differentiated by the incorporation of sulphur-containing ions at various sites in their structure and in different quantities (floridean starch), or of nitrogen-containing ions (cyanophytean starch).

This differentiation in the composition and structure of the main constituents of algae has given rise to enormous variation in their physicochemical characteristics: apart from reactive compounds, susceptible to transformation processes, there are also inactive and resistant compounds. This is the reason why great quantities of algal matter have been preserved in almost their original state in geologically even very ancient sediments.

The diverse composition, structure and physicochemical characteristics of the main constituents of algae, as well as their different quantities, must also be responsible for the oxyreactivity of algal matter.

## 2. Materials and methods

The material was sampled from two different areas on the Polish coast of the Baltic Sea – the Gulf of Gdańsk and the Pomeranian Bay (Fig. 1). Several species of green algae (Chlorophyta), two of brown algae (Phaeophyta) and two of red algae (Rhodophyta) were used in the analysis. One sample, taken in the Świnoujście area, contained a mixture of unindentified algae. For comparison, two samples of sea grass (*Zostera marina* L.) were also taken.

In the oxyreactive TA method the organic substrate sample under investigation is heated at a steady rate in a dynamic atmosphere of air. During the process, the effects of the reactions resulting from the decrease in mass and the absorption or liberation of heat are recorded. These effects should be recorded at high sensitivity and separately at temperature intervals of several degrees. A number of conditions should be fulfilled if the effect of the reaction is to be a function of the structure and composition of the compounds. For example, the reaction surface area of the grains of the material analysed should be of optimum dimensions, the composition of the atmosphere must be stable, and the diffusion of oxygen to the sample and

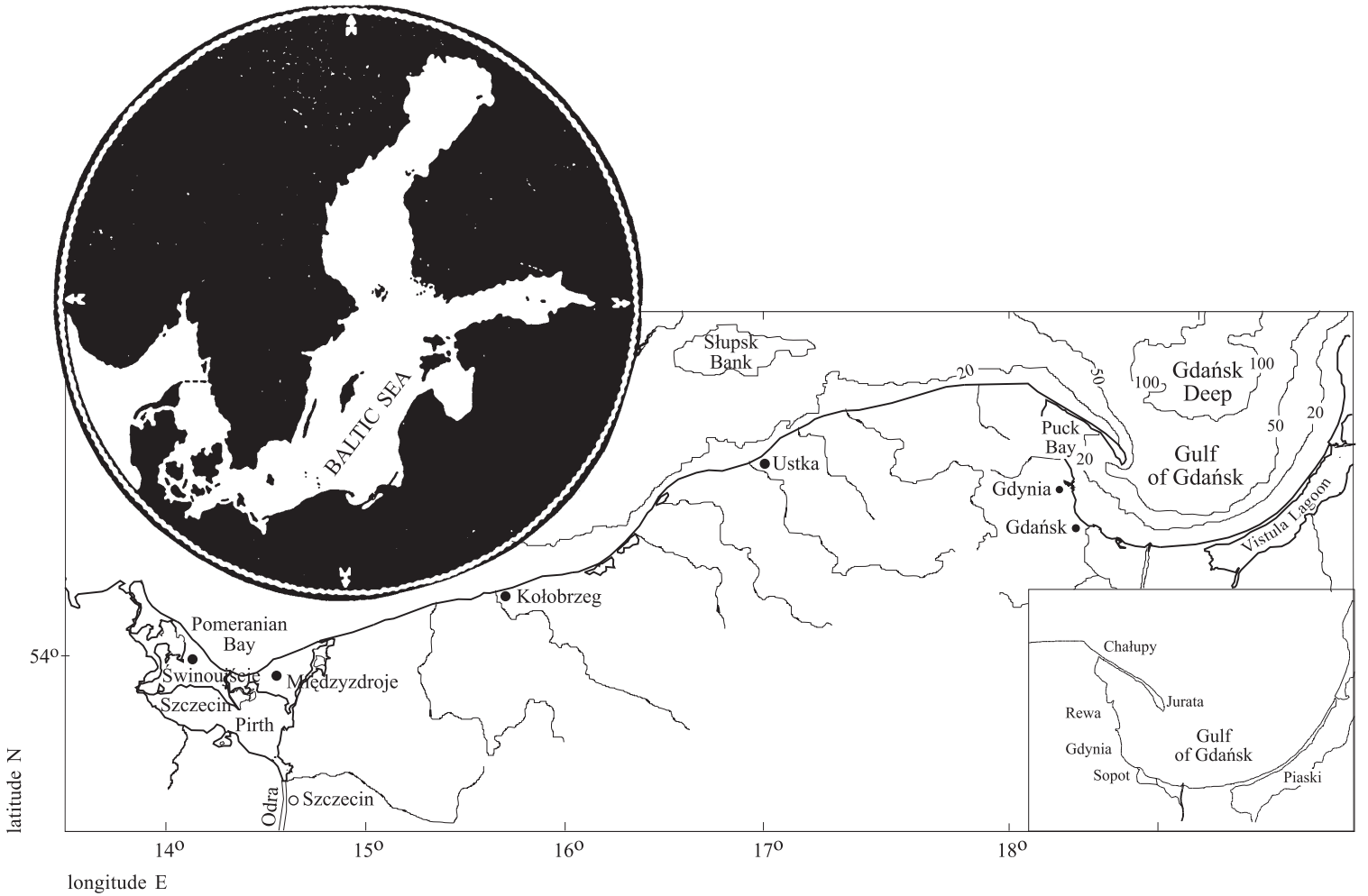


Fig. 1. Map of areas (names of stations used in the text) where material was obtained

the removal of gaseous products should be as rapid as possible. The analytical conditions and preparation of materials will be described in detail in another paper (MS).

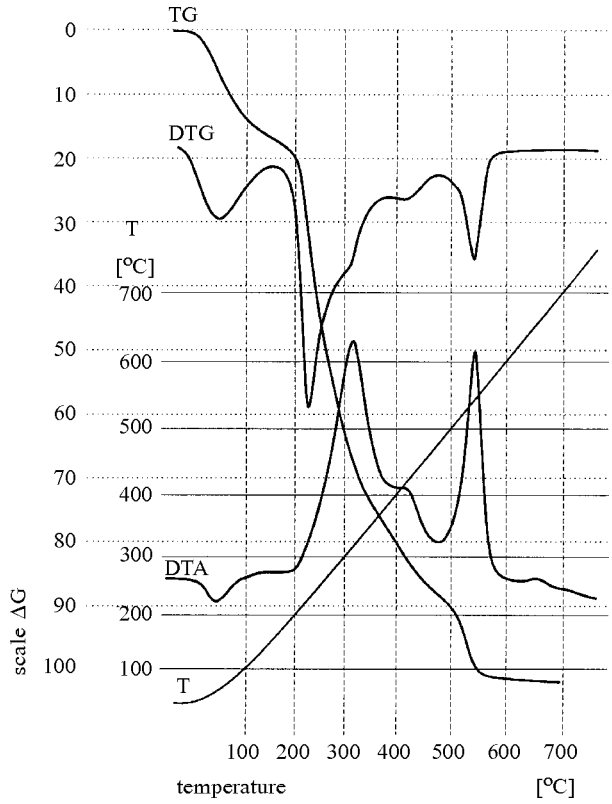


Fig. 2. Example of derivatography analysis (simultaneous – TG, DTG, DTA, T of *Enteromorpha intestinalis* from Rewa station)

The analysis yields a number of interlinked curves (Fig. 2):

- T curve – shows the rise in temperature during the heating process;
- DTA curve – shows the occurrence of reactions associated with heat absorption (down) or heat liberation (up), following a temperature increase (see the T curve). The areas below the DTA curve peaks are proportional to the amount of heat liberated. The top of a peak marks the maximum reaction temperature, which is read off by projecting the peak along the vertical (dashed) lines on the T curve;
- TG curve – shows the diminution in the mass of the sample during heating. The loss can be read off the $\Delta$G scale;

- DTG curve – shows the rate of loss of mass and is obtained by differentiating the TG curve.

A comparison of the DTG and DTA curves illustrates the relations between the heat liberated and the loss of mass.

The graph (Fig. 2) illustrating the analysis of the material selected (*Enteromorpha intestinalis* (L.) Link from the Gulf of Gdańsk) shows the possibilities of interpretation – the characteristics of the DTA and DTG curves are a 'fingerprint' of the oxyreactivity of the samples analysed. The results of such an analysis provide a basis for determining the reaction energies, the quantities of volatile products evolved, the enthalpy change and the specific heats of the substances involved. Comparison of such a graph with the graph of a standard sample enables the structure and composition of the analysed substance to be established.

The TA analysis was carried out in a derivatograph (MOM, Hungary); the container was formed from a set of two small platinum plates produced by ourselves.

The following analytical parameters were used:

- mass of the analytical sample – 80 mg: 50% algal tissue and 50% $Al_2O_3$;
- rate of heating $10^\circ\,\mathrm{min}^{-1}$;
- dynamic air atmosphere.

The accuracy of the analysis will improve if:

- the sample is ground to a grain diameter of less than 0.5 $\mu$m;
- agglomeration of the material during its mixing is avoided;
- the heating chamber is aerated dynamically (suction speed above the container – 4 dm $\mathrm{min}^{-1}$, blowing efficiency below the container – 1.5 dm $\mathrm{min}^{-1}$).

## 3. Results

The results of the oxyreactive thermal analysis (TA) of the algae and sea-grass investigated are presented in Fig. 3 and Fig. 4. Only the DTA and DTG curves have been used in the interpretation. Their shapes are distinctive not only among the divisions of algae but also within them. The sea-grass matter has clearly displayed its individuality.

All the curves have one feature in common: a single, clearly dominant peak, characterising the reactions occurring at temperatures between 230 and 320°C. However, it is due to compounds the chemical composition and structure of which can vary significantly. This variation is manifested by

the peak shape and temperature (max. reaction temperature). Further differences emerge when the peak details of the DTA and DTG curves are analysed, and become even clearer when the interrelations between the peaks are subjected to scrutiny.

To make such a detailed interpretation possible, the DTG peak is shown in Fig. 3 on an enlarged scale. The relative positions of the DTA and DTG peaks indicate whether the dissociation reaction is or is not linked to an oxidation reaction. They therefore show whether, in the decomposition reaction occurring at a certain temperature, products reacting with oxygen are liberated and what the heat of the reaction is. From such an interpretation it can be deduced which functional groups are released during reactions at a given temperature. The reaction temperature reflects to some extent the energy level of the bond that has been broken, and the peak shape is an indication of the homogeneity of this type of bond. Where several different bond types are present, the TA curves reflect the relations between them. This is well illustrated by the DTG peaks of three different algal taxa: *Chaetomorpha* spp., *Enteromorpha clathrata* (Roth) Greville and *Enteromorpha ramulosa* (J. E. Smith) Hooker.

The *Chaetomorpha* are most probably made up of substances with homogeneous bonds, and their decomposition in the 230–320°C temperature range releases products which react with oxygen, as a comparison of the DTA and DTG curves shows. Following this approach, *Enteromorpha ramulosa* contains two, and *Enteromorpha clathrata* three types of bonds which participate in the oxidation and subsequent liberation of gaseous products. In *Enteromorpha ramulosa* it is low-energy bonds that are predominant, *i.e.* they react with oxygen at a lower temperature. In the course of decomposition, dehydration can also take place if OH groups are present in the material.

Analysis of the shape of TA curves at higher temperatures provides further insight into the composition of the samples investigated. However, the scope of this paper limits us to the presentation of only a fraction of the possibilities of TA methods in studies of organic matter.

The different interpretations of DTA and DTG curves discussed above facilitate a short comparative analysis of a number of algal taxa and sea grass.

The highest degree of similarity is seen in the case of two samples of sea grass (*Zostera marina*). The material was taken from two stations distant from each other, but situated in the same water body (Gulf of Gdańsk). The algal species *Enteromorpha intestinalis* from the Gulf of Gdańsk (stations: Jurata and Rewa) behave similarly during thermal analysis. The shapes of the DTA curves are identical. The differences in peak heights are due to

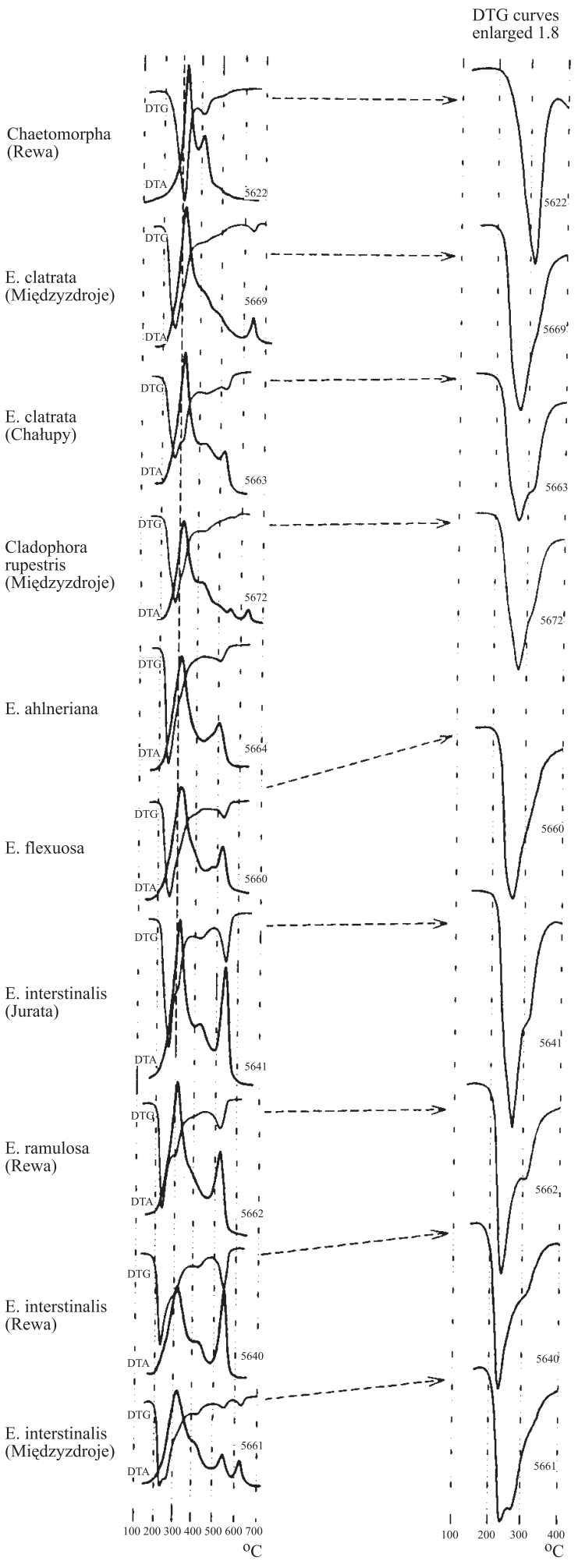


Fig. 3. DTA and DTG curves of different species of Chlorophyta from selected stations (name in parentheses)

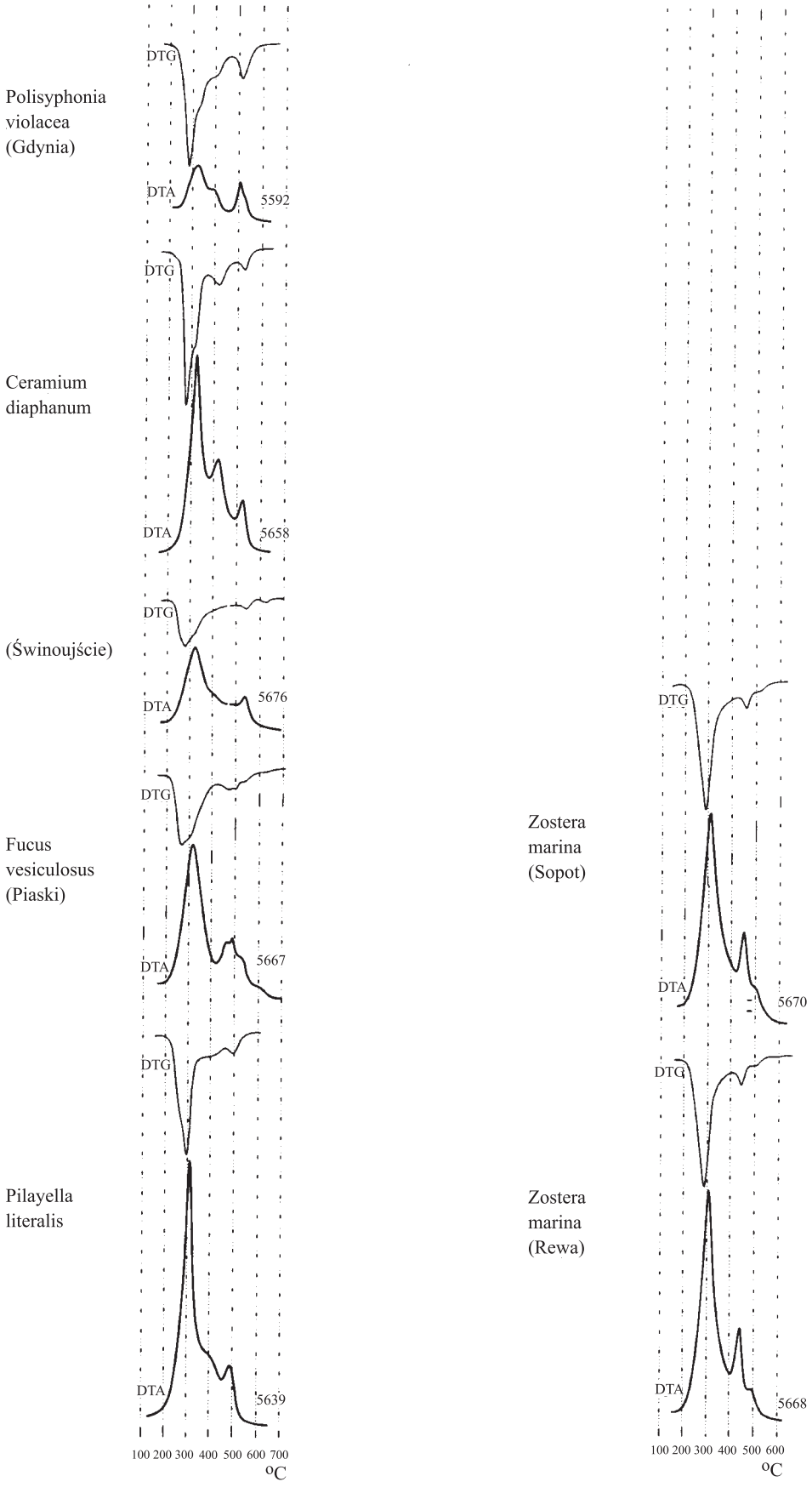


Fig. 4. DTA and DTG curves of different species of Rhodophyta and Phaeophyta, and Zostera marina from selected stations (name in parentheses)

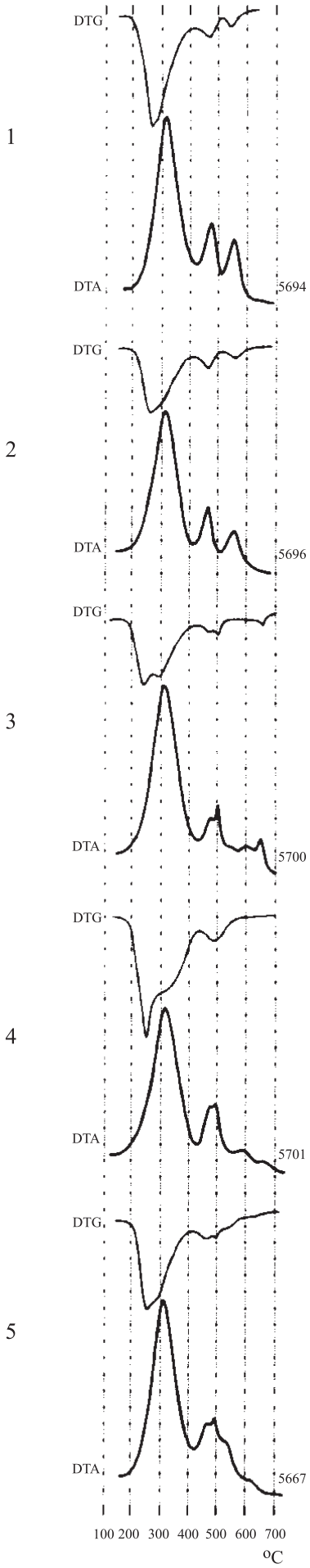


Fig. 5. DTA and DTG curves of Fucus vesiculosus with morphological differentiation: 1 – external part of thallus; 2 – bladder wall; 3 – internal part of thallus; 4 – core; 5 – whole thallus

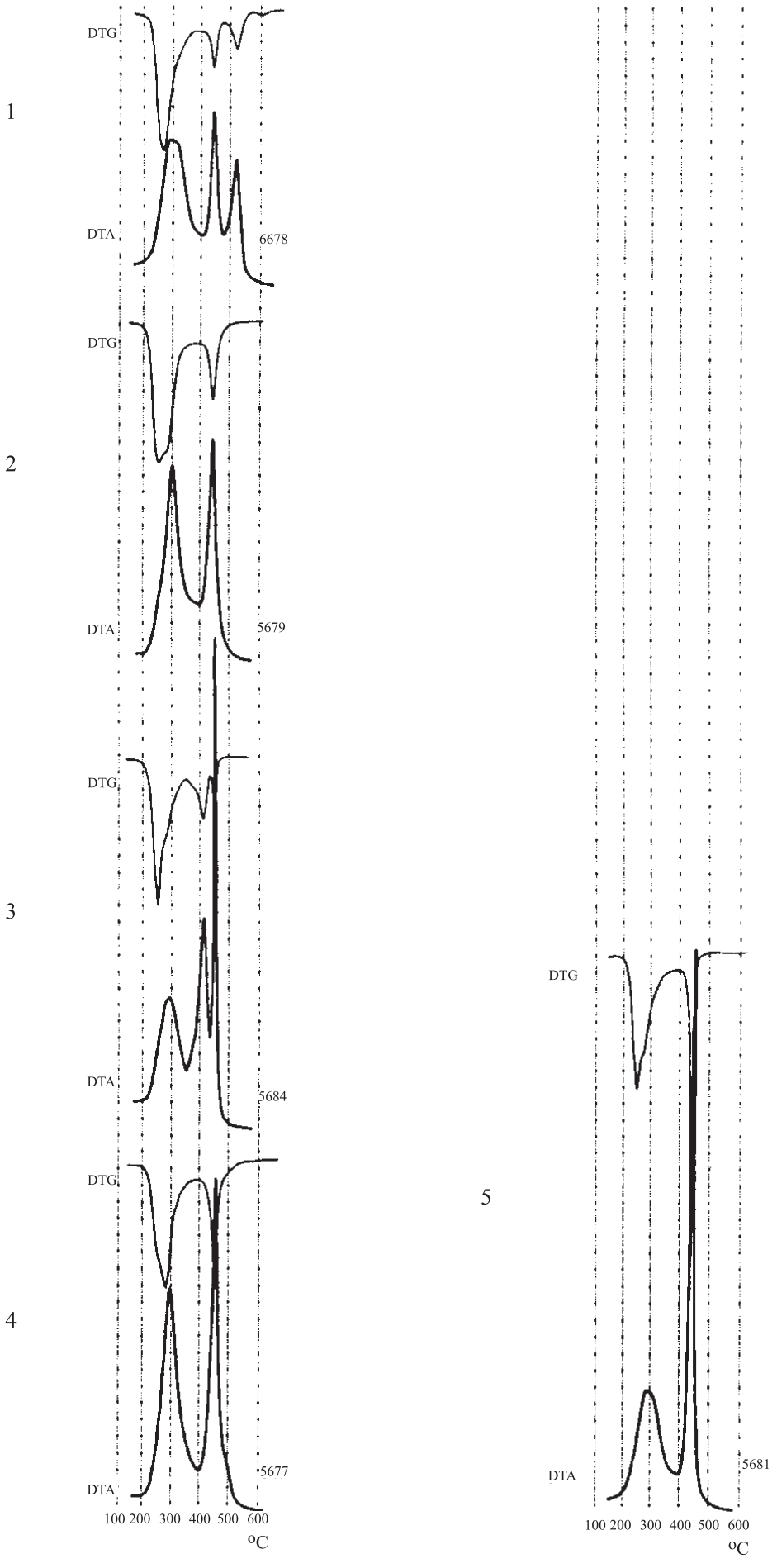


Fig. 6. DTA and DTG curves of Laminaria spp. from Spitsbergen with morphological differentiation: 1 – thin part (white) of thallus; 2 – thick part (brown) of thallus; 3 – core (brown part); 4 – rhizoidum; 5 – core (light-brown porous part)

the fact that before the sample was ground, the grains of sand attached to the dried thallus of the algae sampled in Rewa had not been washed off. This was noticed only during the microscopic examination to find the cause of the elevated quantity of ash in the sample. However, these observations were made after the thermal analysis. It was not possible to repeat this analysis owing to the limited mass of the sample. On the other hand, the differences in shape of the low-temperature DTG peaks may suggest differences in the OH-group content in the sample matter. Perhaps the cause is that the samples were not transported in precisely the same way. During the washing process, quantities of some components like slime, with an elevated OH-group content, may have been washed out.

The shape of the *Enteromorpha ramulosa* curve from Rewa is similar to that on both the *E. intestinalis* curves. The sample shows the same feature – a higher OH-group content – as *E. intestinalis* from Rewa. This would suggest that this feature is an algal property caused by an environmental peculiarity rather than some defect in preparing the samples for analysis. The shapes of the curve of *Enteromorpha intestinalis* from the Pomeranian Bay (Międzyzdroje station) are quite different from those from the Gulf of Gdańsk and are very similar to those of *Cladophora rupestris* (L.) Kutzing and *Enteromorpha clathrata* from Międzyzdroje. The similarity of these three green-algae species sampled from the same station and the above-mentioned differences in *Enteromorpha intestinalis* suggest that the environment exerts a greater influence on the chemical composition of algae than their taxonomical identity does. The curve shapes of two samples of *Enteromorpha clathrata* from Międzyzdroje (Pomeranian Bay) and Chałupy (Gulf of Gdańsk) – two stations several hundred km apart – are very different. The curve shapes of *Enteromorpha ahlneriana* Bliding and *E. flexuosa* J. Agardh are very similar, but different for Chaetomorpha from Rewa (Gulf of Gdańsk). The presence of a peak at 630–640°C in the algal matter of the three different species sampled in the Pomeranian Bay (Międzyzdroje) is confirmation of the close dependence between the chemical composition of algal matter and environmental specificity.

The curve shapes of the four species of red and brown algae studied vary, but are generally different from those of the green algae (Fig. 4). This could confirm the differences in chemical composition between red and brown algae, and green algae. The very high peaks found in *Ceramium diaphanum* (Lightfood) Roth and *Pilayella litoralis* (Lyngbye) Kjellman are interesting. Perhaps these algae contain relatively high-energy matter. *Ceramium* and *Polysiphonia* species have been reported as containing a variety of polysaccharides which give a gelatinous secretion.

The curve shapes of *Fucus vesiculosus* L. are clearly differentiated, depending on which part of the thallus the analytical material was taken from (Fig. 5). The cells of the flattened part are richer in storage substances than those of the stipe or near the stipe part, a fact suggested by the literature data on the physiological role of particular parts of a plant, as well as by the peak sizes. Perhaps these peaks ought to be associated with the great diversity of carbohydrates found in this species (Levring *et al.*, 1969). Of these compounds, *ca* 15% is alginic acid, 9% – mannitol, 9.2% – fructose and – 2.6% laminarin. However, their content varies depending on the area and growing season. According to literature sources, the alginic acid content may be as high as 28%. The variation in curve shape is greater still for different parts of the thallus of Laminariales algae sampled on the Spitsbergen coast (Fig. 6). This example illustrates how useful TA oxyreactive analysis is in algological investigations, and shows that there is a considerably greater diversity in curve shape for certain parts of the algal thallus in species which are anatomically and morphologically highly differentiated.

A comparison of the results of analysing the various parts of *Fucus vesiculosus* and the *Laminaria* shows that only the flattened parts display any similarity – there are three peaks in similar temperature ranges. This leads to the conclusion that the chemical composition of this part in both groups of algae is approximately the same. The variable proportions of the peak heights at different temperatures in the two groups of algae indicate that the chemical constituents are present in variable quantities. The curve shapes of other parts of the two groups of algae are distinctly different.

## 4. Discussion

In order to validate the use of DTA and DTG curves to interpret the variations between the chemical composition and structure of algae, some of the results of the analysis of the model substances – agar-agar, alginic acid, sodium alginate, and microbiological media on an agar base – are now presented. Fig. 7 illustrates the curves of four types of agar-agar produced by different manufacturers. These are agars prepared from red-algae processed in various ways to improve their microbiological utility. Such samples therefore retain the principal properties of the original material but differ in certain features. The fact that the DTA and DTG curves of these agars are very similar is a good recommendation for the use of oxyreactive TA in investigations of biological matter.

Fig. 7 also shows the chemical composition of agar (the main part of the structure). It is a polysaccharide made up of agarose chains to which branched chains of agaropectin are attached (parts of the molecule are shown in Fig. 7). Comparison of the agar-agar curves with those of the investigated

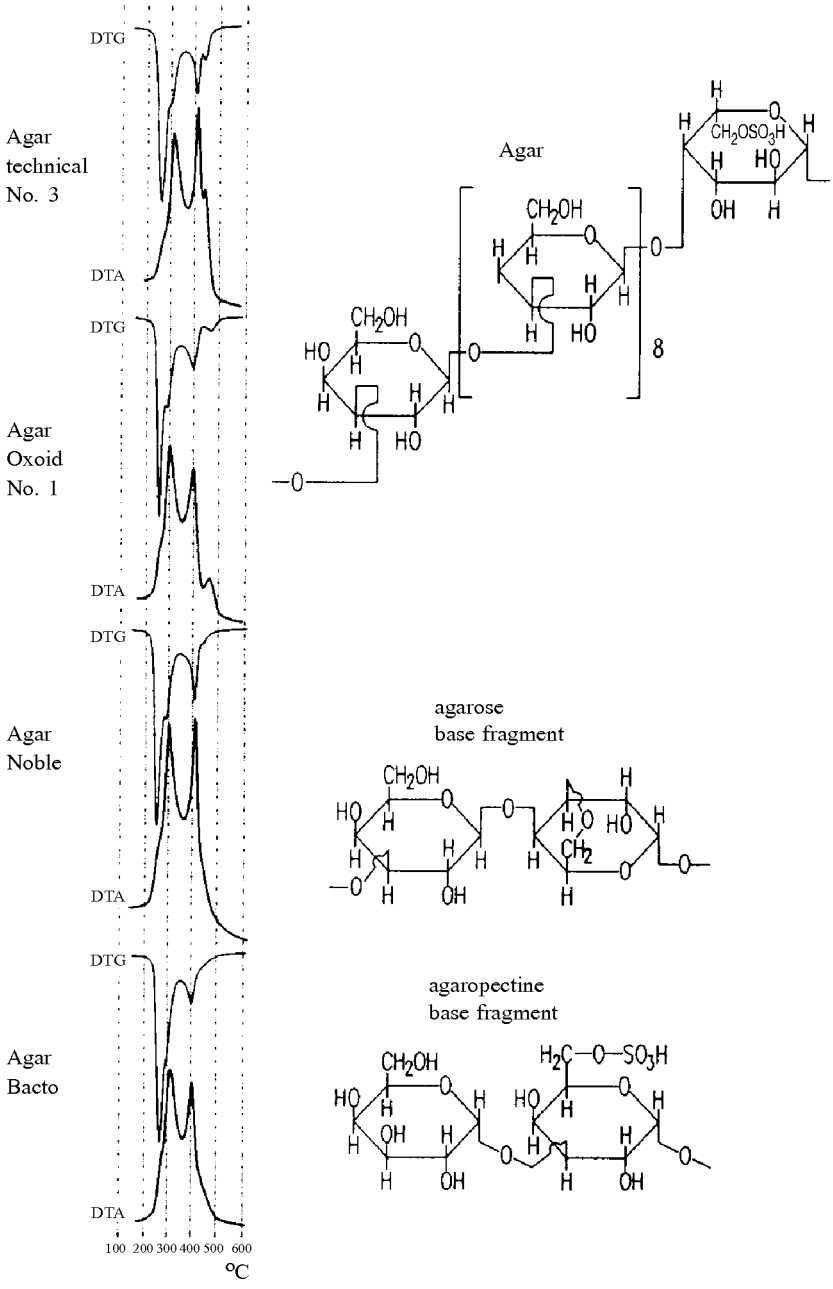


Fig. 7. DTA and DTG curves of different microbiological agars with the characteristics of agar structure

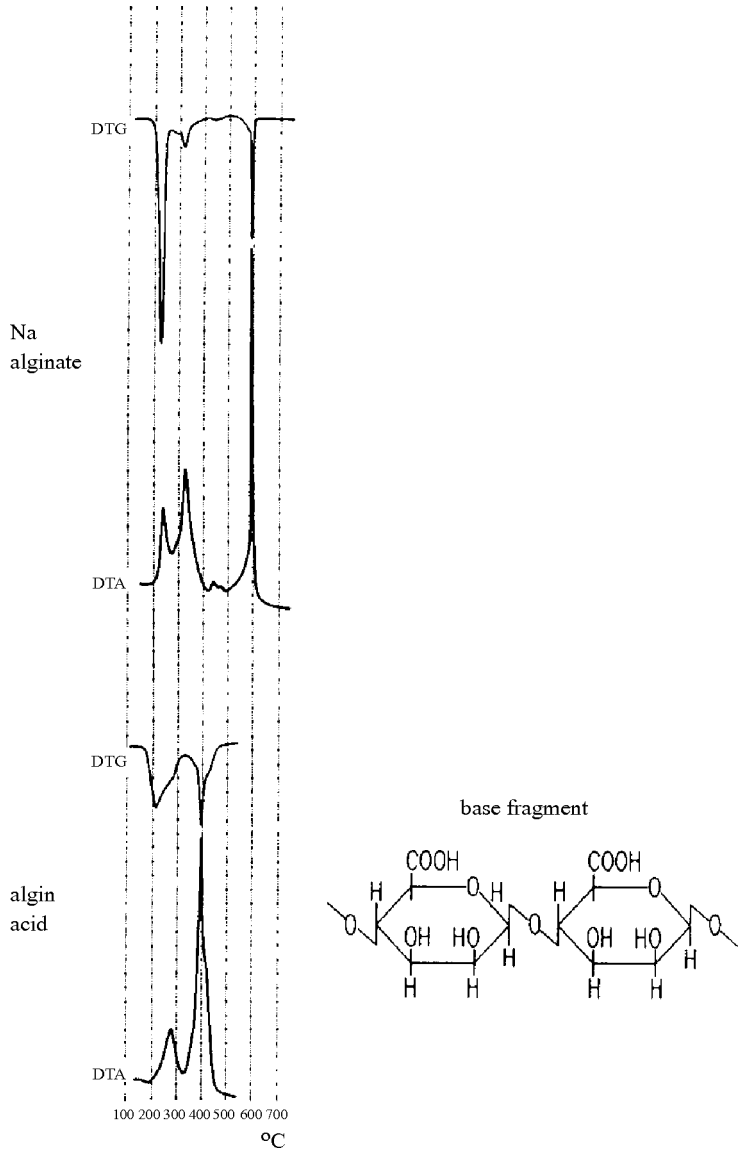


Fig. 8. DTA and DTG curves of sodium alginate and alginic acid

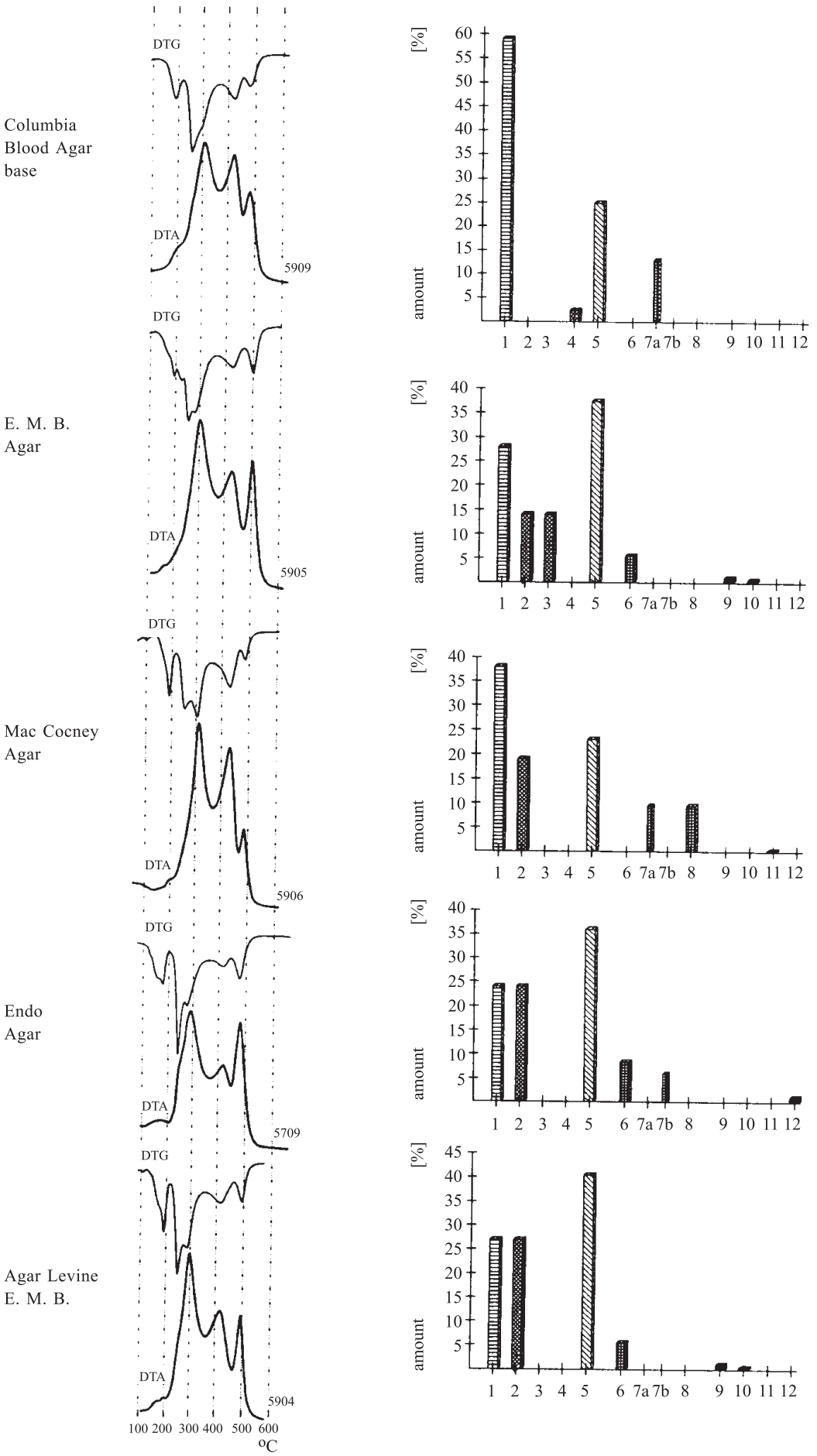


Fig. 9. DTA and DTG curves and composition of the agar-agar used as microbiological medium (1 – peptone; 2 – lactose; 3 – saccharose; 4 – starch; 5 – agar-agar; 6 – phosphate; 7a – sodium chloride; 7b – sodium sulphide; 8 – bile salt; 9 – eosin; 10 – methylene blue; 11 – neutral red; 12 – fuchsin)

algae reveals a striking similarity between them and those of *Ceramium diaphanum* and the *Chaetomorpha*. Such differences as exist emerge from the fact that besides agar, red algae contain other substances, such as cellulose, pectins, chlorophyll and other pigments. Moreover, the variable pectin part is often encrusted with different quantities of calcium.

In order to confirm the presence of another important polysaccharide in the algae investigated, *i.e.* alginic acid, the model preparations of this constituent were also analysed. These comprise two compounds, alginic acid and its derivative sodium alginate (Fig. 8). Sodium alginate is formed by replacing the hydrogen in the carboxyl group (COOH) in the alginic acid with sodium. The proportion of COONa to COOH groups is variable: 1:4, 1:3, 1:2, and sometimes less than 1:4. The sharp peak at 600°C specific to sodium alginate may indicate the presence of this compound in certain parts of the *Laminaria* thallus, especially in the stipe (Fig. 6).

It is very interesting that the curve shapes of some green algae (*Enteromorpha intestinalis*) are similar to those of the microbiological media. In these, the main compounds are agar, lactose (saccharoid) and peptone (protein) (Fig. 9).

This preliminary study shows clearly how the TA curve shapes vary depending on the constitution (origin) of the biological material, and confirms the biochemical diversity of the algae investigated. As the method is repeatable, the results obtained are specific to the material selected for investigation.

The similarity, demonstrated above, between the curves of the algae and the test substances extracted from algae are sufficient proof that the principles set out in this article for interpreting the results of algal TA are valid. TA can be used in taxonomic investigations to establish the systematic membership of certain species of algae based on fragments of thallus. The method can also be applied to specify an environment by differentiating the chemical composition of particular species, and is also suitable for assessing biochemical diversity in different parts of the thallus.

The proposals for the use of TA set out here require further validation, during which it may be possible to find new applications in marine biology.

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