

# Chemical Composition and Biological Activities of the Black Sea Algae *Polysiphonia denudata* (Dillw.) Kütz. and *Polysiphonia denudata f. fragilis* (Sperk) Woronich

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The two investigated algae had almost identical sterol composition, but there were significant differences in the composition of the polar components and especially in the composition of the volatiles. *P. denudata f. fragilis* extracts possessed a stronger biological activity (antibacterial, antifungal and toxicity against *Artemia salina*). Despite the minute morphological differences between the two algae, we recommend *P. denudata f. fragilis* to be regarded as *P. denudata* subsp. *fragilis*.

## Introduction

*Polysiphonia denudata* and its form *P. denudata f. fragilis* are red algae (Rhodophyta) and belong to family Rhodomelaceae, order Ceramiales, class Rhodophyceae. *P. denudata* is a widely spread species, while *P. denudata f. fragilis* is endemic for the Black Sea.

Despite their wide distribution, there is a limited data on the chemical composition of the algae from genus *Polysiphonia*. Until now, mainly polysaccharides (Usov and Ivanova, 1987), lipids (Kamenarska *et al.*, 2001; Kurbanov *et al.*, 1997) and sterols (Elyakov and Stonic, 1988; Al Easa *et al.*, 1995) have been investigated. A number of brominated phenols were identified in the red algae from family Rhodomelaceae (Faulkner, 2001; Flodin and Whitfield, 2000). Only fatty acids of *P. denudata* were reported (Kamenarska *et al.*, 2001; Kurbanov *et al.*, 1997). There are no investigations, concerning the chemical composition and biological activity of *P. denudata f. fragilis*. Recently, we found a significant antiviral activity in extracts from *P. denudata* from the Black Sea (Serkedjieva *et al.*, 2000). So it is of interest to investigate the chemical composition and biological activity of the

two algae. Comparisons of taxonomic significance could also be made.

## Materials and Methods

### Collection of the samples

Sample of *P. denudata* was collected in May, near the town of Ahtopol at the Black Sea.

Sample of *P. denudata f. fragilis* was collected in May, near the village of Varvara at the Black Sea. The distance between both locations is about 12 km.

Voucher specimens were deposited in the herbarium of the Faculty of Pharmacy, Medical University, Sofia, and identified by Dr. Stefka Dimitrova-Konaklieva.

All samples were investigated microscopically. Bacterial or other cells, different from those of the investigated species, were not observed.

### Preparation of the extracts

About 50 g (dry weight) of each sample were consecutively extracted with 700 ml methanol, 700 ml methanol/chloroform (1:1 v/v) and 700 ml chloroform. The extracts were combined and con-

centrated. About 300 ml water was added. The chloroform layer was separated and dried under reduced pressure at 40 °C yielding 2.9 g dry wt. The aqueous residue was extracted twice with 300 ml *n*-butanol, that after elimination of solvents the *n*-butanol extract yielded 3.8 g and the water extract 5.5 g.

#### *Isolation and analysis of sterols*

The chloroform extract of each alga was evaporated under reduced pressure at 40 °C. Part of the dry residue (about 300 mg) was applied to a silica gel (30 g) column. The column was eluted with 100 ml petroleum ether, followed by 100 ml petroleum ether/acetone (15:1 v/v), 100 ml petroleum ether/acetone (10:1 v/v), 200 ml chloroform, 100 ml chloroform + 1% methanol, 100 ml chloroform + 3% methanol, 100 ml chloroform/methanol (4:1 v/v), 100 ml chloroform/methanol (3:1 v/v), 100 ml chloroform/methanol (1:1 v/v), 200 ml methanol. Fractions containing sterols were identified by thin layer chromatography (TLC). Further purification by prep. TLC on silica gel G (hexane/acetone; 10:1 v/v) was performed. The total sterol mixtures were investigated by GC and GC/MS.

Quantitative analysis was performed on a Pye Unicam 304 gas chromatograph equipped with FID and a capillary column SPB-1 (30 m × 0.32 mm, 0.25 µm film thickness), at 230 °C and programmed to 300 °C at 4 °C min<sup>-1</sup> and 10 min hold. Injector and detector were at 300 °C.

For GC/MS was used a Hewlett Packard 6890 + MS 5973 instrument equipped with a capillary column SPB-50 (30 m × 0.32 mm, 0.25 µm film thickness). The MS source was at 250 °C and the ionisation voltage at 70 eV. The GC oven temperature was at 270 °C and programmed to 290 °C at 4 °C min<sup>-1</sup> and 20 min hold.

#### *Isolation and analysis of volatiles*

Dry residue of the chloroform extracts (250 mg from *P. denudata* and 500 mg from *P. denudata f. fragilis*) were subjected to a four-hour distillation-extraction in a Lickens-Nickerson apparatus. The volatile compounds were extracted from the distillate with diethyl ether (yield: *P. denudata* – 8.1 mg, 3.2% from the chloroform extract), *P. denudata f. fragilis* – 17.1 mg, 3.4% from the chloro-

form extract), and investigated by GC/MS equipped with a capillary column HP5-MS (30 m × 0.25 mm, 0.25 µm film thickness), at 40 °C and programmed to 280 °C at 6 °C min<sup>-1</sup>.

#### *Isolation and analysis of the components of n-butanol extract*

##### *Isolation of α-O-methylanosol*

Part of the dry *n*-butanol extract from *P. denudata f. fragilis* (0.95 g), dissolved in MeOH was chromatographed on Sephadex LH-20 column (2 × 100 cm) eluted with methanol (2 ml.min<sup>-1</sup>). The main fraction (180 mg) was identified as 3,4-dibromo-5-methoxymethyl-1,2-benzenediol (α-O-methylanosol) (MS, UV, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra identical with literature data) (Kubo *et al.*, 1990).

##### *GC/MS of the n-butanol extract*

5 mg of each *n*-butanol extracts were subjected to a silylation with 50 µl abs. pyridine and 75 µl of bis(trimethylsilyl)trifluoroacetamide (BSTFA). The mixtures were heated at 80 °C for 30 min and analysed by GC/MS equipped with a capillary column HP-5 (23 m × 0.2 mm, 0.5 µm film) at 100 °C and programmed to 315 °C at 5 °C min<sup>-1</sup> and 10 min hold.

##### *Identification of compounds by GC-MS analyses*

The identification was accomplished using computer searches on a NIST98 MS Data library. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass-spectral fragmentation. When possible reference compounds were co-chromatographed to confirm GC retention times especially when isomeric compounds have similar spectra.

##### *Antibacterial test*

For the investigation of the antibacterial activity we used a modification of bioautography, recently developed in our laboratory (Kujumgiev *et al.*, 1993). *Staphylococcus aureus* 209 and *Escherichia coli* WF+ were used as test microorganisms. For each test 0.5 mg (dry wt.) of each extract was used (chloroform, butanol, water). The samples were dissolved in 0.1 ml ethanol and placed in 25 ml of

agar in Petri dish with  $d = 10$  cm. The antibacterial activity was measured by the diameter of the inhibitory zones in the soft agar layer after a 72-hr incubation at 37 °C. An inhibitory zone with a diameter of less than 10 mm indicated lack of activity.

#### Antifungal test

For the investigations we used the agar cup method (Spooner and Sykes, 1972). Parts of the investigated extracts (0.5 mg dry wt. dissolved in 0.1 ml ethanol) were placed in a well with a diameter of 10 mm,  $10^7$  cells (*Candida albicans* 562) were used per Petri dish ( $d = 10$  cm) with 25 ml of agar. After incubation at 37 °C for 72 hrs the diameters of the inhibition zones were determined. A diameter of less than 10 mm indicated lack of activity.

#### Cytotoxicity assay

*Artemia salina* lethality (Solis et al., 1993) was determined using caffeic acid phenethyl ester (CAPE) as active reference substance. Concentrations of 1000, 100, 10 and 1 ppm were used; 10 *A. salina* per concentration plus control.

## Results and Discussion

#### Biological activity

The Gram (+) bacteria *Staphylococcus aureus*, the Gram (-) bacteria *Echerichia coli* and the fungi *Candida albicans* were used for the investigation of the anti-microbial and antifungal activity. The chloroform, *n*-butanol and water fractions, as well as the volatile compounds were tested. The results are reported in Table I. All fractions, except the water extracts from the two algae were active against *S. aureus*. The anti-microbial activity is stronger in *P. denudata f. fragilis*. Only the *n*-butanol fraction from *P. denudata f. fragilis* showed an activity against *E. coli*. The chloroform, butanol and water fractions from *P. denudata f. fragilis* and the butanol extract from *P. denudata* showed an activity against *C. albicans*. The water fraction from *P. denudata* was not active at all and the water fraction from *P. denudata f. fragilis* showed an activity only against *C. albicans*. The activity of  $\alpha$ -O-methyllosol (one of the important constituents of the *n*-butanol extract of *P. denudata f. fragilis*) was also tested. It appears to be

Table I. Antibacterial and antifungal activity of extracts from *P. denudata* and *P. denudata f. fragilis*.

Extracts from:	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
	Diameter of the inhibitory zone [mm] <sup>a</sup>		
<i>P. denudata</i>			
Chloroform extract	17.3 ± 0.6	0	0
Butanol extract	22.3 ± 0.6	0	17.7 ± 0.6
Water extract	0	0	0
Volatile compounds	15.3 ± 0.6	0	0
<i>P. denudata f. fragilis</i>			
Chloroform extract	20.7 ± 1.2	0	19.7 ± 0.6
Butanol extract	24 ± 1.0	14 ± 0	18.7 ± 1.2
Water extract	0	0	22.7 ± 0.6
Volatile compounds	15.7 ± 0.6	0	0
$\alpha$ -O-Methyllosol	27 ± 1.0	16.7 ± 1.2	23.3 ± 1.5

<sup>a</sup> Diameter of the inhibition zone less than 10 mm means absence of activity.

higher than the activity of the total butanol extract.

The cytotoxicity of the extracts was also investigated by using brine shrimp (*Artemia salina*) test. The results are reported in Table II. The *n*-butanol extract of *P. denudata f. fragilis* showed a strong cytotoxic activity.

Table II. Cytotoxicity assay of *P. denudata* and *P. denudata f. fragilis* extracts.

Sample	LC <sub>50</sub> [ $\mu$ g/ml]
<i>P. denudata</i>	
Chloroform extract	> 1000
Butanol extract	384 ± 55
Water extract	> 1000
<i>P. denudata f. fragilis</i>	
Chloroform extract	162 ± 16
Butanol extract	109 ± 19
Water extract	> 1000
$\alpha$ -O-methyllosol	60.3 ± 18
CAPE (standard)	0.45 ± 0.5

$\mu$ g is the amount of the dry residue of the extracts.

#### Investigation of the chloroform extract.

The chloroform extract was subjected to a further fractionation and two fractions were obtained: sterols (by column chromatography, followed by preparative TLC) and volatile

compounds (by distillation-extraction in a Likens-Nickerson apparatus).

### Sterol composition

Sterols are important for the functioning of the cell membranes. Their composition depends on the environment and on the specificity of the organism and in some cases is used for chemotaxonomic investigations. The sterols were investigated by GC/MS (qualitative analysis) and by GC (qualitative and quantitative analyses). The data obtained is reported in Table III. It is evident that the sterol composition of both algae investigated is characteristic for the red algae. Cholesterol, characterised by  $^1\text{H-NMR}$ , MS and RT, is the main sterol constituent. It is accompanied by low concentrations of other sterols, containing additional double bonds at C-22 and/or alkylation at C-24.  $^1\text{H-NMR}$  of the total sterol mixture showed unambiguously that the  $\Delta^{22}$ -sterols contain only 22Z-double bond. Such unusual sterols were recently found in Black Sea invertebrates (De Rosa *et al.*, 1999; Slantchev *et al.*, 2000). Till now there are no data about the presence of such sterols in algae. There are no significant differences between sterols from *P. denudata* and *P. denudata f. fragilis*. Only one investigation on sterols from *Polysiphonia* sp. (*P. brodiae* and *P. ferulavea*) from Qatar (Al Easa *et al.*, 1995) was reported, and their composition strongly differs from those of the Black Sea species. The content of cholesterol was lower in the algae from Qatar, while the alkylated sterols, especially those containing 29 carbon atoms and those with C-22 double bond, were signifi-

cantly higher. Contrary to some other red algae, no 5 $\alpha$ -stanols were found in the two species analysed. Recently, we found that compared to other *Polysiphonia* species some constituents of the cell membranes (phospholipids and glycolipids) of *P. denudata* also show significant differences in their composition (Kamenarska *et al.*, 2001). Probably the changes in the environment have a strong effect on the composition of the lipid cell membranes. All investigated *Polysiphonia* sp. were collected at different locations and that could explain the differences in the cell membrane constituents.

### Volatiles

The volatile constituents, which are a part of the chloroform extract, are of interest because they possess an average antibacterial activity (Table I). It is known that volatile compounds from plants often contain defensive compounds, attractants, repellents, antifeedants, insecticides, etc. There is a limited number of publications on volatiles from algae (Kamenarska *et al.*, 2000, Mahran *et al.*, 1993, Gally *et al.*, 1993) and no investigations on the volatiles from *Polysiphonia* species. Volatiles from algae are also of importance because they are continually emitted in the atmosphere.

We analysed the volatiles from both algae by GC/MS. The results are summarised in Table IV. The composition of the volatiles was very complex, and there are significant differences between the two samples.

There are a large number of aldehydes in *P. denudata f. fragilis* while in *P. denudata* only two aldehydes were found. Some of the aliphatic aldehydes identified were similar to those, forming the so-called "green odour", which attract some pollinators (Wang *et al.*, 1999). Probably such compounds in algae can take part in the relationships between marine organisms.

Hydrocarbons were found in the volatiles of both algae, but their amount was more than twice higher in *P. denudata*. Saturated *n*-paraffins predominated. Heptadecane was the main hydrocarbon in both algae. Low concentrations of three olefins were detected. With the exception of phenanthrene all other hydrocarbons appeared to possess straight chains. Phenanthrene was found in unusually high concentrations for algae. Earlier, a brominated dihydrophenanthrene was found in *Polysiphonia ferulaceae* (Aknin and Samb, 1992).

Table III. Sterol composition (% from the total sterol fraction)\*.

Sterols	<i>P. denudata</i>	<i>P. denudata f. fragilis</i>
(22Z)-Cholesta-5,22-dien-3 $\beta$ -ol	0.4	0.6
Cholesterol	83.3	80.1
24-Methyl-cholesta-5,22Z-dien-3 $\beta$ -ol	3.3	0.7
24-Methyl-cholesta-5,24(28)-dien-3 $\beta$ -ol	1.9	3.4
24-Ethyl-cholesta-5,22Z-dien-3 $\beta$ -ol	0.3	0.9
24-Ethyl-cholest-5-en-3 $\beta$ -ol	0.7	0.6
Fucosterol	0.7	–

\* Values obtained from three parallel measurements. The standard deviations (related to peak proportion on the chromatograms) are as follows:  $\pm 0.3$  for cholesterol and  $\pm 0.1$  for the others.

Table IV. Volatile compounds from *P. denudata* and *P. denudata f. fragilis* (% from the total volatiles)\*.

Compounds	<i>P. denudata</i>	<i>P. denudata f. fragilis</i>
<b>Aldehydes</b>		
Hexanal	0.1	0.2
Heptanal	–	< 0.1
2,4-Heptadienal	–	< 0.1
Decanal	–	< 0.1
2,4-Decadienal	–	< 0.1
Benzaldehyde	< 0.1	< 0.1
Benzeneacetaldehyde	–	< 0.1
<b>Acids</b>		
Butanoic acid	< 0.1	–
Hexanoic acid	< 0.1	–
Pelargonic acid	< 0.1	–
Myristic acid	4.6	2.2
Palmitic acid	5.9	3.4
<b>Esters</b>		
Benzylbenzoate	1.0	–
Isopropylmyristate	0.4	–
5-Oxopentanoic acid, methyl ester	–	0.4
Pentanedioic acid, dimethyl ester	–	< 0.1
Heptanedioic acid, dimethyl ester	–	< 0.1
9-Oxononanoic acid, methyl ester	–	0.1
Octanedioic acid, dimethyl ester	–	0.2
Nonadioic acid, dimethyl ester	–	0.1
Lauric acid, methyl ester	–	0.1
12-Methyltridecanoic acid, methyl ester	0.8	–
Myristic acid, methyl ester	–	11.5
Pentadecanoic acid, methyl ester	–	0.7
16:3 acid, methyl ester	–	0.6
(9,12) 16:2 acid, methyl ester	–	< 0.1
16:1 acid, methyl ester	–	13.6
16:1 acid, methyl ester (isomer)	–	0.4
14-methylpentadecanoic acid, methyl ester	< 0.1	–
Palmitic acid, methyl ester	–	31.0
17:0 acid, methyl ester	–	0.1
Linolenic acid, methyl ester	–	0.8
Linoleic acid, methyl ester	–	2.8
18:1 acid, methyl ester	–	2.9
18:1 acid, methyl ester (isomer)	–	1.8
Stearic acid, methyl ester	–	0.6
(7,10,13)20:3 acid, methyl ester	–	0.5
<b>Hydrocarbons</b>		
Tetradecane	0.2	0.1
Pentadecane	0.3	0.2
1-Hexadecene	0.6	–
Hexadecane	0.8	0.1
1-Heptadecene	0.2	0.1
Heptadecane	7.3	2.5
1-Octadecene	–	0.1
Octadecane	0.8	0.2
Eicosane	0.4	–
Phenantrene	0.5	0.3

Table IV (continued).

Compounds	<i>P. denudata</i>	<i>P. denudata f. fragilis</i>
<b>Terpenes</b>		
Dihydroactinidiolide	0.5	< 0.1
Isophytol	2.6	–
$\beta$ -Ionone	–	< 0.1
Farnesylacetone	0.4	–
<b>Aromatic compounds</b>		
Phenol	–	< 0.1

\* The ion current generated depends on the characteristics of the compound and is not a true quantitation. This method is suitable for comparing the chemical composition of different organisms, because the deviations caused by the differences in the intensity of the mass spectral fragmentation will be identical.

Contrary to most of the terrestrial plants where the concentration of terpenoids is high in the volatiles from algae it is low. We found a relatively high concentration of terpenoids in *P. denudata*, while in *P. denudata f. fragilis* they were in traces. Dihydroactinidiolide and hexahydrofarnesylacetone are characteristic for most of the marine algae investigated (Kamenarska *et al.* 2000, Sakagami *et al.*, 1991).  $\beta$ -Ionone was also found earlier in algae (Rzama *et al.*, 1995). It is a strong deterrent against some insects (Wang *et al.*, 1999) and probably might be active against marine Arthropoda.

We found small amounts of free fatty acids. The free fatty acids are active metabolites. They serve as an important energetic substrate for the cells. Hydrocarbons and free fatty acids, which are common components of the volatiles, often serve as sex pheromones and allelopathic substances (Yasumoto *et al.*, 2000). Very often it is not clear whether they are natural or produced by some hydrolysis during the purification procedures. The method used was a soft one and no hydrolysis occurred, so the acids identified are natural components of the investigated algae. Their concentration and diversity is higher in *P. denudata*.

Phenol was found in low concentrations in the volatiles from *P. denudata f. fragilis*. It might be one of the antibacterial constituents. Terpenes and free fatty acids also possess a mild antibacterial activity.

The main components in the volatiles of *P. denudata f. fragilis* appeared to be esters. While in *P. denudata* there are only three esters, in *P. denudata*

*f. fragilis* there is a large diversity of fatty acid methyl esters (FAME). Although their origin is often disputed (native or artefacts) some of the methyl esters in *P. denudata f. fragilis*, could be of interest, because dioic and oxoacids are rarely found in algae. Two esters of branched fatty acids were detected only in *P. denudata*.

#### Investigations of polar constituents

Most of the polar constituents extracted with *n*-butanol possess hydroxyl, amine and carboxyl groups, which determine their polarity. Silylation can transform these compounds into ethers, possessing enough volatility to be investigated by GC/MS. The results obtained are summarised in Table V.

Acids, carbohydrates and bromine-containing compounds are the main polar constituents in both algae. Monocarboxylic acids (fatty acids and benzoic acid) were present only in *P. denudata f. fragilis*, while their oxidised derivatives (polycarboxylic acids and hydroxylated fatty acids) were concentrated almost entirely in *P. denudata*. Probably the enzymes, responsible for the oxidation of the fatty acids are much more active in *P. denudata* than in *P. denudata f. fragilis*. Free amino acids predominated in *P. denudata*.

The carbohydrates appeared as a complex mixture in which only glucose and fructose were identified.

Many bromophenols have been previously identified in other algae of the family Rhodomelaceae (Faulkner, 2001; Flodin and Whitfield, 2000).

GC/MS spectra showed the presence of a big number of brominated compounds in both algae investigated. According to their mass spectral fragmentation most of the compounds are bromophenol derivatives of the benzyl alcohol. Such compounds are of interest, because some halogenated compounds possess antibacterial, antifungal, antiviral activities, etc. (Kubo *et al.*, 1990). Other polar components of the investigated algae with antibacterial activity were fatty acids, especially hydroxylated ones and one ester of phosphoric acid.

In order to investigate the composition of the bromine derivatives, we subjected the total butanol extract from *P. denudata f. fragilis* to a column chromatography on Sephadex LH-20. One crystal-

Table V. Composition of the *n*-butanol extracts from *P. denudata* and *P. denudata f. fragilis* (% from the total silylated compounds in the extract)\*.

Compounds	<i>P. denudata</i>	<i>P. denudata f. fragilis</i>
<b><i>Br-containing compounds</i></b>	<b>8.8</b>	<b>7.3</b>
Monobromo compounds	1.0	0.5
Dibromo compounds	7.8	6.8
<b><i>Hydroxy acids</i></b>	<b>3.7</b>	<b>0.3</b>
Hydroxyacetic acid	0.2	–
2-Hydroxypropanoic acid	2.6	0.2
3-Hydroxypropanoic acid	0.1	–
2,3-Dihydroxypropanoic acid	0.7	0.1
Hydroxymalic acid	< 0.1	–
2,3,4-Trihydroxybutyric acid	0.1	–
<b><i>Polycarboxylic acids</i></b>	<b>4.3</b>	<b>0.2</b>
1-Propene-1,2,3-tricarboxylic acid	2.0	–
Butanedioic acid	1.6	0.1
Citric acid	0.7	0.1
<b><i>Monocarboxylic acids</i></b>	<b>–</b>	<b>1.2</b>
Hexadecanoic acid	–	0.7
Oleic acid	–	0.2
Octadecanoic acid	–	0.1
Benzoic acid	–	0.2
<b><i>Amino acids</i></b>	<b>2.6</b>	<b>0.8</b>
Alanine	0.1	–
Proline	< 0.1	–
Glycine	< 0.1	–
Glutamine	0.2	–
5-Oxoproline	2.3	0.8
<b><i>Esters</i></b>	<b>1.2</b>	<b>0.2</b>
Trimethylphosphate	0.4	–
2,3-Dihydroxyhexadecanoic acid, propyl ester	–	0.2
Myristic acid, 2,3-bis-hydroxypropyl ester	0.8	–
<b><i>Sugars</i></b>	<b>4.3</b>	<b>0.6</b>
Glucose	3.8	0.6
Fructose	0.5	–
<b><i>Polyols</i></b>	<b>1.0</b>	<b>0.3</b>
Glycerol	1.0	0.3
<b><i>N-containing compounds</i></b>	<b>2.6</b>	<b>–</b>
Uridine	2.4	–
2H-purin-6-amine	0.2	–

\* The ion current generated depends on the characteristics of the compound and is not a true quantitation.

line substance was isolated. Its mass spectrum was characteristic for a dibromo compound ( $[M]^+$  at 310, 312, 314, rel. int. ratio 1:2:1). The  $^1\text{H-NMR}$  spectrum showed signals for one aromatic proton ( $\delta$  6.94, s, 1H), a methoxy group ( $\delta$  3.32, s, 3H) and a benzylic methylene group ( $\delta$  4.28, s, 2H). This data indicated that the compound must be a dibromodihydroxy derivative of benzylmethyl ether.



After comparison of its MS, UV,  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra with literature data (Kubo *et al.*, 1990) it was identified as  $\alpha$ -O-methylanosol (3,4-dibromo-5-methoxymethyl-1,2-benzenediol). This compound has been found in different *Polysiphonia* species but not in *P. denudata* (Gribble, 1996). This compound appears to be the main component of the *n*-butanol extract from *P. denudata f. fragilis*.  $\alpha$ -O-methylanosol is suggested to be a represen-

tative of a new class of plant growth regulators showing gibberelin-like activity; and could also be used as an antifouling agent (Kubo *et al.*, 1990).

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