1.80 (8H, *m*, $-CH_2-$), 2.07 (3H, *s*, C-4 Me), 2.32 (2H, *t*, $J = 7$ Hz, H-6'), 2.46 (2H, *t*, $J = 7$ Hz, H-1'), 3.66 (3H, *s*, OMe). EIMS (probe) 70 eV, *m/z* (rel. int.): 255 $[M+1]^+$ (6), 224 (10), 223 $[M-MeO]^+$ (80), 208 (24), 204 (17), 194 (20), 180 $[M-C_3H_6O_2]^+$ (15), 177 (25), 176 (14), 166 (13), 163 (12), 150 (45), 149 (29), 148 (14), 135 (14), 129 (29), 126 (68), 122 (10), 121 (17), 107 (14), 98 (26), 97 (41), 95 (13), 93 (17), 91 (11), 88 (11), 87 (21), 84 (16), 83 (12), 81 (21), 79 (26), 77 (13), 74 $[C_3H_6O_2]^+$ (100).

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# WAX COMPOSITION OF *SARGASSUM FULVELLUM**

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**Key Word Index**—*Sargassum fulvellum*; Sargassaceae; Phaeophyta; wax; 5-methylhexyl esters; 2-ethylhexyl esters; 5-methylhexanol; 2-ethylhexanol.

**Abstract**—Sixty-seven compounds were characterized in the wax of *Sargassum fulvellum*. Characteristic components were the 5-methylhexyl esters of octanoic, decanoic, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic, and the 2-ethylhexyl esters of the same acids. The wax of *S. fulvellum* contains hydrocarbons (1.6%), esters (21.8%), free acids (74.9%) and free alcohols (0.3%). The principal free alcohols range in chain length only from $C_6$ to $C_7$.

## INTRODUCTION

*Sargassum fulvellum* (Japanese name, 'Hondawara') is an annual seaweed which is used in folk medicine and for food, but the wax constituents have not so far been studied. Other waxes and constituents of the *Sargassum* genus have been studied: sargasterol from *S. ringgoldianum* [1], sarganan and sarganol from *S. natuns* [2], alginate and alginic acid from *S. swartzii*, *S. johnstonii* and *S. tenerrinum* [3], fucosterol and saringosterol from *S. ringgoldianum* [4]. In this paper, the wax components from *S. fulvellum* are reported.

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## RESULTS AND DISCUSSION

The fronds of *S. fulvellum* were collected from the seashore in Kushikino-shi, Kagoshima, Japan, in August 1979. The dried alga was chopped finely and extracted with $CH_2Cl_2$ for 90 days at room tem-

Table 1. Constituents identified in wax oil of *S. fulvellum*

| Peak No. | Compound | % | Peak No. | Compound | % |
|---|---|---|---|---|---|
| 1 | Tetradecane | 0.2 | 45 | Methyl linolate | 0.2 |
| 1′ | 5-Methyl hexanol | | 46 | Lauric acid | 0.9 |
| 2 | Methyl octanoate | 0.1 | 47 | Methyl linolenate | 0.1 |
| 3 | 2-Ethyl hexanol | 0.2 | 48 | 2-Ethylhexyl myristate | 0.2 |
| 5 | Pentadecane | tr | 49 | Heptacosane | 0.1 |
| 6 | Octanoic acid | 0.1 | 50 | 5-Methylhexyl palmitate | 1.1 |
| 7 | Hexadecane | tr | 51 | 5-Methylhexyl palmitoleate | 1.7 |
| 9 | Heptadecane | 0.1 | 52 | Octacosane | tr |
| 10 | Methyldecanoate | 0.2 | 53 | Myristic acid | 5.8 |
| 11 | Octadecane | 0.1 | 54 | 2-Ethylhexyl palmitate | 2.5 |
| 13 | Nonadecane | 0.1 | 55 | 2-Ethylhexyl palmitoleate | 1.8 |
| 14 | Methyl undecanate | 0.1 | 56 | Nonacosane | tr |
| 15 | Eicosane | 0.2 | 57 | *iso*-Pentadecanoic acid | 2.0 |
| 17 | Methyl laurate | 0.2 | 58 | Pentadecanoic acid | 0.6 |
| 18 | 5-Methylhexyl octanoate | tr | 59 | Triacontane | tr |
| 20 | 2-Ethylhexyl octanoate | 0.1 | 60 | 5-Methylhexyl stearate | 0.1 |
| 21 | Heneicosane | 0.2 | 61 | 5-Methylhexyl oleate | 1.5 |
| 22 | Methyl tridecanate | 0.1 | 62 | 5-Methylhexyl linolate | 0.1 |
| 23 | Docosane | 0.1 | 63 | Palmitic acid | 24.2 |
| 24 | Methyl myristate | 1.2 | 63′ | 5-Methylhexyl linolenate | |
| 26 | Methyl pentadecanate | 1.7 | 64 | 2-Ethylhexyl stearate | 13.5 |
| 27 | 5-Methylhexyl decanoate | tr | 64′ | Palmitoleic acid | |
| 28 | 2-Ethylhexyl decanoate | 0.1 | 65 | 2-Ethylhexyl oleate | 2.2 |
| 30 | Tricosane | 0.1 | 66 | 2-Ethylhexyl linolate | 0.2 |
| 32 | Tetracosane | 0.1 | 67 | 2-Ethylhexyl linolenate | 0.1 |
| 33 | Methyl palmitate | 3.1 | 68 | Heptadecanoic acid | 1.1 |
| 34 | Methyl palmitoleate | 1.1 | 70 | Stearic acid | 1.7 |
| 35 | Decanoic acid | 0.8 | 71 | Oleic acid | 17.2 |
| 36 | 5-Methylhexyl laurate | 0.1 | 72 | Linoleic acid (all *cis*) | 2.8 |
| 37 | Pentacosane | 0.1 | 73 | Linoleic acid | 2.4 |
| 39 | Undecanoic acid | 0.6 | 74 | Linolenic acid | 1.2 |
| 40 | 2-Ethylhexyl laurate | 0.2 | | | |
| 41 | Hexacosane | 0.1 | | | |
| 42 | Methyl stearate | 0.3 | | | |
| 43 | Methyl oleate | 1.4 | | | |
| 44 | 5-Methylhexyl myristate | 0.2 | | | |

The relative concentrations (%) were calculated on the basis of GC peak areas. tr; trace.

perature. The wax (0.66%: $d_4^{25}$, 0.8170; $n_D^{25}$, 1.4753; $[\alpha]_D^{25}$, +22.1°; A.V., 70.8; S.V., 89.9) obtained by evaporation of the solvent under $N_2$ was separated into neutral and fatty acid fractions by aqueous sodium carbonate. Each fraction was separated by CC on Si gel and the individual subfractions analysed by GC/MS. The 52 components listed in Table 1 were identified. Peak numbers show the elution order on the Thermon-600T column. The fatty acid fraction examined after esterification was considerably less complex than the wax. All $C_4$–$C_{18}$ saturated and unsaturated normal fatty acid were found in this fraction. Peak No. 63 is the main component and peaks Nos 6, 35, 39, 46, 53, 57, 58, 64′, 68, 70, 71, 72, 73 and 74, which are fatty acids, are considerably smaller in amount. The neutral fraction was chromatographed on Si gel and divided into five fractions (see Experimental). The hexane fraction has peak Nos 1, 5, 7, 9, 11, 13, 15, 21, 23, 30, 32, 37, 41, 49, 52, 56 and 59. Among these, we find the $C_{14}$–$C_{30}$ alkanes. The benzene and ether fractions contained fatty acid esters; peak Nos 2, 10, 14, 17, 22, 24, 26, 33, 34, 42, 43, 45 and 47 were identified as methyl esters of $C_8$–$C_{18}$ fatty acids. The peak Nos 20, 28, 40, 48, 54, 55, 64, 65, 66 and 67 consist of characteristic components of the algal wax, and were identified as 2-ethylhexyl esters by comparison of their MS spectra with those of synthetic samples. In the same way, the MS spectra of peak Nos 18, 27, 36, 44, 50, 51, 60, 61, 62 and 63′ showed the presence of 5-methylhexyl esters. These compounds were identical with synthetic samples, prepared by esterification of the requisite fatty acid with 5-methylhexanol synthesized from isoamyl bromide. The ethyl acetate subfraction of the neutrals contained peak Nos 1′ and 3. With regard to peak No. 1′, we found it was 5-methyl hexanol, and MS spectra showing *m/z* 166 ($M^+$, 3.0%), 98 ($M^+$–$H_2O$, 25.0%), 70 ($M^+ - 46$, 100%), and 56 ($M^+ - 60$, 73.3%), and it was further identified by comparison of its $t_R$ and MS spectra with that of a synthetic sample. In the same way, the MS spectra of No. 3 showed 130 ($M^+$, 3.0%), 112 ($M^+ - H_2O$, 25.0%), 98 ($M^+ - 32$,

Table 2. Composition of wax fraction *S. fulvellum*

| No. of carbon | % Fraction [I] | [II] | [III] | [IV] |
|---|---|---|---|---|
| 6 | — | — | — | — |
| 7 | — | — | — | 24 |
| 8 | — | — | 1 | 75 |
| 9 | — | 1 | — | — |
| 10 | — | — | 1 | — |
| 11 | — | 1 | — | — |
| 12 | — | 1 | 2 | — |
| 13 | — | 1 | — | — |
| 14 | 5 | 1 | 8 | — |
| 15 | 6 | 6 | 6 | — |
| 16 | 6 | 8 | 44 | — |
| 17 | 6 | 19 | 7 | — |
| 18 | 6 | 1 | 30 | — |
| 19 | 7 | 9 | — | — |
| 20 | 9 | 1 | — | — |
| 21 | 9 | 1 | — | — |
| 22 | 8 | 1 | — | — |
| 23 | 8 | 11 | — | — |
| 24 | 8 | 19 | — | — |
| 25 | 6 | 8 | — | — |
| 26 | 5 | 12 | — | — |
| 27 | 5 | — | — | — |
| 28 | 4 | — | — | — |
| 29 | 4 | — | — | — |
| 30 | 2 | — | — | — |
| 31 | — | — | — | — |

[I] Hydrocarbons; [II] esters; [III] free acids; [IV] free alcohols.

50.2%), and 83 ($M^+ - 47$, 100.0%), identical to that of an authentic sample. Thus, the wax from *S. fulvellum* was mainly composed of free fatty acid (74.9%) and esters (21.8%) of fatty acids and aliphatic alcohols with smaller quantities of several hydrocarbons (1.6%) and free alcohols (0.3%). There were no halogenated components. The most noteworthy feature of this wax is the presence of 5-methylhexanol and 5-methylhexyl esters. The 5-methylhexyl esters consisted of seven saturated and three unsaturated acid moieties which represented 4.8% in wax. There are also 10 equivalent 2-ethylhexyl esters which amount to 7.4% of the wax. The abundance of free fatty acids was greater than in *Asparagopsis taxiformis* [5]. The compositions of the fractions are listed in Table 2. A wide range of chain lengths are present in the hydrocarbons, $C_{14}$–$C_{30}$, and in the esters, $C_9$–$C_{26}$. Free acids, though great in quantity, have a small range of chain lengths with $C_{16}$ and $C_{18}$ as the major components; unusual amounts of odd-numbered free acids are also present.

## EXPERIMENTAL

*Gas chromatography.* Wax components were analysed by means of a Shimadzu Model GC-MINI-2 equipped with a flame ionization detector (FID). A 0.3-mm i.d. × 40 m glass capillary column coated with Thermon-600T was used. The oven temp. was maintained at 100° for 10 min and then programmed to 210° at a rate of 5°/min. $N_2$ was used as a carrier gas at a flow rate of 0.5 ml/min through the column with a split ratio of 1:70.

*GC/MS.* A Shimadzu Model LKB-9000B mass spectrometer was used. The GC column conditions were the same as described above. Other operating parameters were as follows; column temperature, programmed from 60 to 210° at 2°/min; carrier gas, He; ionizing voltage, 70 eV; accelerating voltage, 3200 V; ion source temp., 210°.

*Preparation of free acid and neutral fraction.* The wax (2.00 g) was dissolved in $Et_2O$ and then was added to 5% $Na_2CO_3$. After shaking, the aq. layer (free acids) was sepd, acidified with 5% $H_3PO_4$, and again extracted with $Et_2O$. After evaporation of the solvent *in vacuo*, the residue was obtained as yellow liquid (1.50 g). The neutrals (0.40 g) were obtained by evaporation of the original extracting solvent.

*Column chromatography of the neutrals.* Neutrals (0.40 g) were chromatographed on Si gel (2.5 cm × 60 cm) with hexane, $C_6H_6$, $Et_2O$, EtOAc and MeOH, given five fractions; 0.03 g, 0.21 g, 0.11 g, 0.02 g and 0.01 g, respectively.

*Identification of the components in the neutral and free acid fractions.* The components of each fraction were identified by comparing their GC and GC/MS data with those of authentic reference compounds. The free acids were first treated with ethereal $CH_2N_2$ overnight.

*Synthesis of 5-methylhexanol.* The compound was synthesized via a conventional Grignard reaction between isoamyl magnesium bromide and ethylene oxide. The reaction was carried at 10° over 4–6 hr and the product recovered in 60% yield by fractional distillation.

*Synthesis of 5-methylhexyl esters.* Esterification of the

fatty acid (500 mg) with 5-methylhexanol (1000 mg) with a drop of conc. $H_2SO_4$ for 3 hr at 150° gave the corresponding esters (950 mg).

*Synthesis of 2-ethylhexyl esters.* Esterification of the fatty acids with 2-ethylhexanol [3] as above gave the corresponding esters.


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# CONSTITUENTS OF THE ESSENTIAL OIL OF *ARTEMISIA REHAN*

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**Key Word Index**—*Artemisia rehan*; Asteraceae; essential oil; davanone; eudalene; GC/MS analysis.

**Abstract**—The composition of the essential oil of *A. rehan* has been studied. A total of 22 components were identified, the major components being davanone and camphor, and the occurrence of eudalene is noted for the first time. The characteristic blue colour of the oil is due to chamazulene.

## INTRODUCTION

*Artemisia rehan* Chiov is an odorous herb which is widespread in and native to Ethiopia. Although numerous reports appear in the literature on the chemistry of different species of *Artemisia* no studies have been reported on *A. rehan*. In this paper we report our findings on the chemical constituents of the oil obtained by steam distillation of the whole plant.

## RESULTS AND DISCUSSION

The essential oil from the whole plant, obtained by steam distillation of fresh material, was separated on silica gel with hexane and ethyl acetate to separate the hydrocarbons from the oxygenated derivatives. The fractions were then subjected to GC and GC/MS. Identification was confirmed by comparison of $R_t$ values with those of standard compounds as well as by computerized matching of acquired mass spectra with stored NBS mass spectral library in the data system of the GC/MS. A total of 22 components representing 87% of the oil were identified (see Table 1). Among the main components, davanone **1** and chamazulene **2** were isolated by CC and identified on the basis of IR, $^1$H NMR and mass spectra. The spectra obtained were identical with literature values [1, 2] for these compounds. The results of the analysis of the essential oil show that camphor (24%) and davanone (44%) are the major constituents of the oil. Davanone **1** is also reported to occur in the oil of *A. pallens* [3, 4] and in one of the genotypes of *Tanacetum vulgare* [5]. Capillary GC/MS analysis of the oil showed the presence of two additional components (0.6% and trace) which gave very similar mass spectra to that of the major constituent, davanone. These are designated in the table as davanone-isomer-1 and davanone-isomer-2. It is not entirely clear whether these are diastereomeric compounds or closely related structures such as artemone **3**. It has been shown that synthetic davanone diastereomers can be separated on a capillary column

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