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A NOVEL TYPE OF GLYCERIDES BEARING A METHACRYLIC ACID MOIETY FROM THE BROWN ALGA, SARGASSUM FULVELLUM

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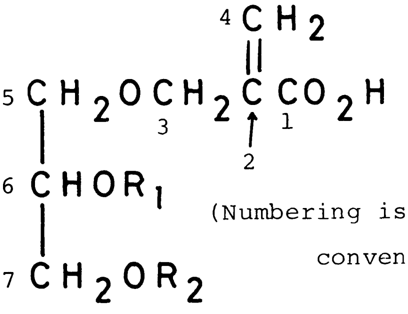
From *S. fulvellum*, a new type of glycerides bearing a methacrylic acid moiety were isolated, and the structures were determined to be 2-[3-eicosadi(and tetra)enoyloxy-2-hydroxypropanoxy)methylpropenoic acids.

Brown algae belonging to *Sargassum* are among the most abundunt seaweeds growing along Japanese coast. The constituents of the *Sargassum* algae have recently been studied, and isolation of unique plastoquinones,[1] chromenols,[1,2] linear terpenoids,[3] and an antibacterial cyclopentenone[4] and lactam[5] has been reported.

In the course of our study on the constituents of *S. fulvellum* (Hondawara in Japanese), we found that the methanol extract of this alga gave a fraction, whose NMR spectrum showed signals characteristic of terminal methylene protons. We seeked to isolate the "terminal methylene compound", and the present report deals with our finding that the compound is a quite new glyceride bearing a methacrylic acid moiety.

The methanol extract of *S. fulvellum* (7 Kg, collected at Awakominato, Chiba, in May, 1980) was concentrated into an aqueous residue, which was successively extracted with hexane and ether. The ether extract was concentrated to give a brown oil (4.2 g). Column chromatography of this residue on silica gel (Merck, Kieselgel 60, Art. 7734) with dichloromethane-methanol (9:1) yielded a fraction (2.8 g), the ^{1}H-NMR spectrum of which showed a couple of broad singlets at δ 6.4 and 5.9 ascribable to the terminal methylene protons. Isolation of the "terminal methylene compound" was extremely difficult, because the more it was purified, the more sensitive to air and light it became. Purification by preparative TLC was fruitless, because the compound changed to an unknown compound on the TLC plate. Isolation and purification were successfully achieved by repeated flash chromatography[6] (Wakogel C-300, hexane:ethyl acetate=2:3) to give an oily substance (200 mg), showing a single spot on TLC (Merck, Kieselgel 60, F254,chloroform:methanol=10:1).

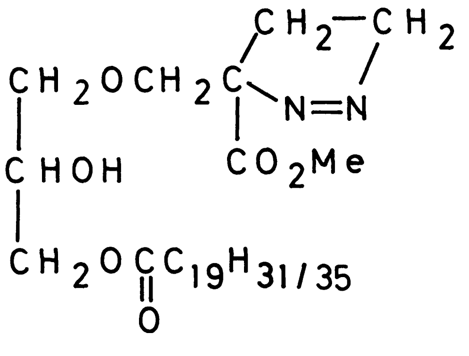


(Numbering is for convenience.)

$\underline{1}$ $\underline{a}$; R_1 = H, R_2 = $C_{19}H_{31}CO$
$\underline{b}$; R_1 = H, R_2 = $C_{19}H_{35}CO$

$\underline{2}$; R_1 = H, R_2 = H
(fulvellic acid)

$\underline{3}$; R_1 = Ac, R_2 = $C_{19}H_{31/35}CO$



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The IR spectrum of this substance, $[\alpha]_D^{25}$ +2.5° ($CHCl_3$,c=0.1), showed the bands at 3600-2200, 1690 (conjugated CO_2H), 1730 (CO_2R), and 1620 ($=CH_2$) cm^{-1}. Its ^1H-NMR spectrum (100 MHz,$CDCl_3$) exhibited a set of signals due to an unsaturated fatty acid moiety at δ 5.3 (br.t,CH=CH), 2.80 (br.s,$C=C-CH_2-C=C$), 2.32 (t,$CH_2\underline{CH_2}-C=O$), 2.00 (m,$CH_2\underline{CH_2}-C=C$), 1.64 (m,CH_2 β to C=O), 1.25 (br.s, CH_2's), 0.90 (deformed t,CH_3). Also it showed poorly resolved multiplets in the region of 4.3-5.5 ppm, the pattern of which closely resembled that appearing in the ^1H-NMR spectrum of glycerine-1-stearate except a singlet at δ 4.20. The signals of the terminal methylene protons appeared at δ 6.41 (br.s) and 5.95 (br.s). The chemical shifts and the shapes of these two signals were reminiscent of those of the terminal methylene protons of dimethyl itaconate (δ 6.31 and 5.70).

Detailed inspection of the 360 MHz ^1H-NMR spectrum[7] ($CDCl_3$) revealed that the substance had the structure $\underline{1}$. All the protons belonging to the glycerine moiety formed the second-ordered coupling system [δ 4.171 (2H,H-7,$\underline{AB}X$,J_{AB}=9.9Hz,J_{AX}=5.9 Hz,J_{BX}=2.9Hz), 4.054 (1H,H-6,$AB\underline{X}$,pseudoquintet), 3.573 (2H,H-5,$\underline{AB}X$,J_{AB}=9.9Hz,J_{AX}=6.3Hz,J_{BX}=4.5Hz)]. The methylene protons at C- 4 appeared as a pair of doublets

(J=1.2Hz) at δ 5.970 and 6.444, both of which were sharpened by irradiating the singlet at δ 4.222 (2H,H-3), establishing the allylic relationship of these two methylene groups.

Attempts to produce 2 by hydrolysis of 1 failed because of the fragility of the methacrylic acid moiety. Treatment of 1 with diazomethane gave a pyrazoline 4, δ ($CDCl_3$) 3.67 (3H,s,$COOCH_3$), 4.47 (2H,t,J=8Hz). Acetylation of 1 with acetic anhydride in pyridine gave rise to an acetate 3, IR($CHCl_3$) 1735 and 1690 cm^{-1}. Although this acetate showed a single spot on TLC after purification by column chromatography, two acetyl signals appeared in the ^1H-NMR[7] (360 MHz,$CDCl_3$) spectrum; δ 2.078, 2.080 (3H,$COCH_3$), 3.652 (2H,d,H-5,J=5.4Hz), 4.264 (2H,ABX,H-7,J_{AB}=11.3Hz, J_{AX}=5.9Hz,J_{BX}=4.1Hz), 5.22 (1H,pseudoquintet,H-6), 5.961 (1H,d,H-4,J=1.4Hz), and 6.436 (1H,d,H-4,J=1.4Hz). This indicated that 1 was a mixture of 1a and 1b. Separation of 1a and 1b was unsuccessful.

In order to verify the carbon skeleton of 1, the compound 8 was synthesized, and the ^{13}C-NMR spectra of 3 and 8 were compared (Table 1). Etheration of the acetonide 5 with the bromide 6[9] and sodium hydride in dimethylformamide produced

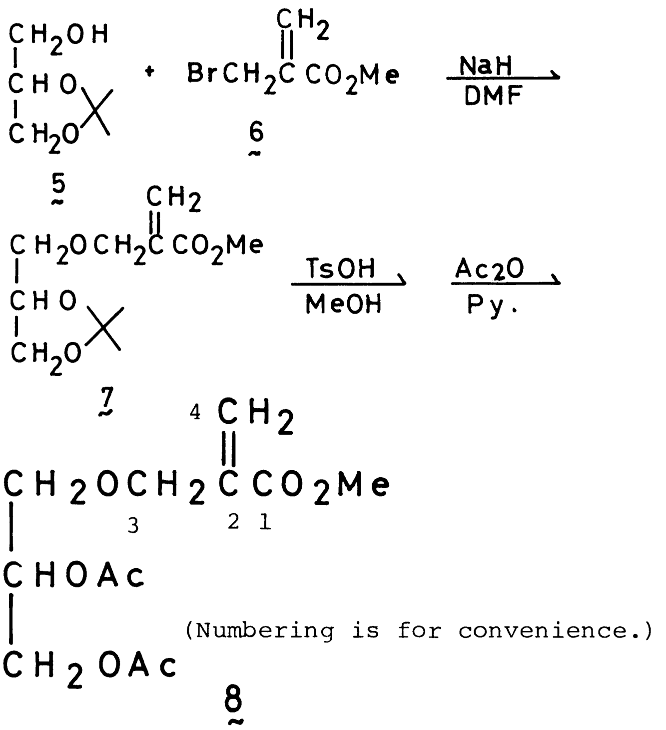

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TABLE 1. ^{13}C-NMR chemical shifts of compounds 3 and 8.

	3	8
C-1	170.46	166.02
C-2	136.41	136.69
C-3	69.28*	68.90**
C-4	128.22	126.06
C-5	62.67	62.72
C-6	70.42	70.17
C-7	69.37*	69.54**

*,** Chemical shifts are interchangeable.

7. The acetonide group of 7 was cleaved by acidic methanol, and the resulting diol was acetylated to give the diacetate 8, ^{1}H-NMR ($CDCl_3$) δ 2.02 (3H,s,Ac), 2.08 (3H, s,Ac), 3.47 (2H,d,H-5,J=6Hz), 3.68 (3H,s,CO_2Me), 4.20 (2H,d,H-3,J=1Hz), 4.20 (2H, ABX,H-7,J_{AB}=12Hz,J_{AX}=4Hz,J_{BX}=6Hz), 5.18 (1H,quintet,H-6), 5.86 (1H,splitted s,H-4), 6.30 (1H,splitted s,H-4). The chemical shifts of the carbons 1-7 of the diacetate were reasonably correspondent to those of 3, establishing the skeleton of 1. We named the compound 2, the basic component of 1, fulvellic acid.

The high resolution mass spectrum of 1 showed a molecular peak of 1a at m/e 504.307 ($C_{29}H_{44}O_7$), and a fragment at m/e 448.317 ($C_{27}H_{44}O_5$), formed from 1b ($C_{29}H_{48}O_7$;m/e 508 in the low resolution MS) by loss of acetic acid. The composition of the fatty acid portion of 1 was confirmed by the following experiment: Treatment of 1 in benzene with a catalytic amount of methanolic m-trifluoromethylphenyltrimethylammonium hydroxide[10] afforded a mixture of methyl esters. GC-MS analysis of this mixture revealed that it consisted of mainly two esters (90% of the ester fraction), $C_{19}H_{31}COOCH_3$ (m/e 318) and $C_{19}H_{35}COOCH_3$ (m/e 322) (3:1), which were derived from 1a and 1b, respectively.

Among the *Sargassum* algae that we investigated, *sagamianum*, *ringgoldianum*, *piluliferum*, *giganteifolium*, *yendoi*, *hemiphyllum*, and *patens* were found to contain this new type of glyceride, although *Hizikia fusiforme*, which was taxonomically close to *Sargassum* algae, did not produce the glyceride as 1. Chemotaxonomical and also biological significance of this "unusual fat" is of interest.

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