two polymers appear to be compostionally similar.

Registry No. HMF, 67-47-0; D-glucose, 50-99-7; D-frustose, 57-48-7; glycine, 56-40-6; methionine, 63-68-3.

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Insect Antifeeding Azulene Derivative from the Brown Alga *Dictyota dichotoma*

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The volatile components of the brown alga *Dictyota dichotoma* obtained by steam distillation of the fresh plants revealed an antifeeding activity to the larva of the cotton leaf worm *Spodoptera littoralis*. 1-(1,3,4,5,6,7-Hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)ethanone was isolated from the crude volatile mixture by chromatographic technique and possessed the antifeeding activity of the mixture.

Many terrestrial plants have been shown to have insecticidal activities (Schildknecht, 1981; Kubo et al., 1982; Bernays and De Luca 1981). However, very few reports can be found in the literature dealing with pesticidal or insecticidal activities of marine plants or algae despite the fact that it has been estimated that the area of marine seaweed reefs is comparable to that of all cultivated land on earth (Moore, 1977).

Our interest in algal insecticidal natural products was initiated by our observation that certain species of algae collected from the Meditteranean sea in Alexandria, Egypt, when left to air-dry had not been infested with house flies. This observation was persistent throughout the drying period of 4 days. When the volatile components of the brown alga *Dictyota dichotoma* obtained by steam distillation of the freshly collected plants was examined for its insecticidal activity, it was found that the crude mixture possessed some insecticidal activity on house flies, cotton leaf worm, and rice weevil; however, it showed a significant antifeeding activity against cotton leaf worm *Spodoptera littoralis*.

The active antifeedant component in the crude volatile mixture was isolated by chromatographic techniques and identified to be 1-(1,3,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)ethanone.

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MATERIALS AND METHODS

Isolation of the Antifeeding Component. The brown alga *D. dichotoma* (1 kg) collected from shallow water along the Mediterranean sea coast of Alexandria, Egypt, during the springs of 1980 and 1981 was washed and cleaned and then subjected to steam distillation. Extraction of the distillate with ether gave 2.6 g of a dark brown essential oil (0.26% yield). The essential oil was chromatographed on a column containing preactivated silica gel (AR-100 mesh, Mallinckrodt, Inc., St. Louis, MO) packed with hexane. Major column fractions were eluted by using hexane followed by a mixture of 5%, 10%, and 20% ether in hexane, acetone, and finally methanol. Column fractions were monitored for antifeeding activity and analyzed by thin-layer chromatography (TLC) and by capillary GC. The most active fraction was further fractionated and purified by preparative TLC. The chemical structure of the active component was identified by spectroscopic techniques.

Instruments and Conditions. Gas chromatography/mass spectrometry (GC/MS) was carried out using a Finnigan 4530 system equiped with a 30-m (0.25 mm i.d.) DB1 fused silica capillary column. Helium was used as the carrier gas and methane as the makeup gas for chemical ionization runs. Chromatographic conditions were as follows: injection port and interface temperature 250 °C;

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Table I. Antifeeding Activity of Crude Essential Oil and Fractions

	% of eaten disks			
	4 h		24 h	
test sample	control	sample	control	sample
crude essential oil	50	5	100	10
hexane fraction	45	38	100	85
5% ether fraction	48	00	100	00
10% ether fraction	45	20	100	50
20% ether fraction	41	40	100	100
acetone fraction	50	55	100	100
methanol fraction	46	50	100	100

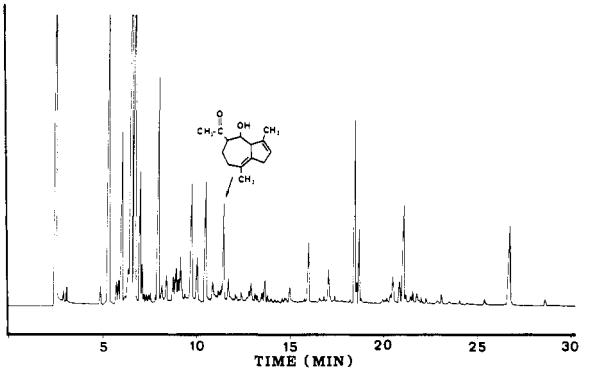

Figure 1. Capillary column gas chromatogram of the steam distillate from the brown algae *Dietyota dichotoma*.

column temperature, 170 °C for 5 min and then 5 °C/min programmed to 250 °C. All spectra in the EI mode were acquired at 70 eV. Infrared (IR) spectra were recorded on a Pye-Unicam 1000 spectrophotometer. Proton magnatic resonance (^{1}H NMR) was recorded with a Varian EM-390 90-MHz NMR spectrometer in $CDCl_3$. Thin-layer chromatography was carried out using silica gel plates of 0.25-mm thickness with the solvent system hexane–ether–acetic acid (90:10:1), and spots were detected after spraying with anisaldehyde reagnet and heating at 100 °C (Stahl, 1973). Preparative TLC was carried out using 1-mm silica gel plates developed 3 times with hexane. Spots were visualized under UV light and eluted in acetone.

Antifeeding Bioassay. The leaf disk method described by Kubo and Nakanishi (1977) was used as an antifeeding test to the insect. Leaf disks (3.14 cm^2) of the castor bean leaves punched out with a cork borer were dipped in the acetone solution of the test samples (1000 ppm) for 3 s. The solvent was removed by evaporation, and control disks were prepared by dipping in acetone for 3 s. A leaf disk coated with the test sample, a control disk, and a fourth instar larva of cotton leaf worm *S. littoralis* were placed in a same Petri dish (7.5 cm) and the temperature was kept at 25 °C. After 4 h and after 24 h the disks were removed and weighed to determine the percentage eaten by the larvae. An average of 10 larvae per each test sample was calculated.

RESULTS AND DISCUSSION

Castor bean leaf disks coated with the crude essential oil revealed an antifeeding activity to the cotton leaf worm. The larvae did not eat the sample disks but ate at least half of the control leafs. Fractions of the crude essential oil obtained by column chromatography showed varied degrees of activity; however, the column fraction eluted in 5% ether in hexane was the most active. This column fraction was further purified by preparative TLC to isolate the active compound. The results of the antifeeding activity of the crude and of the fractions are listed in Table I.

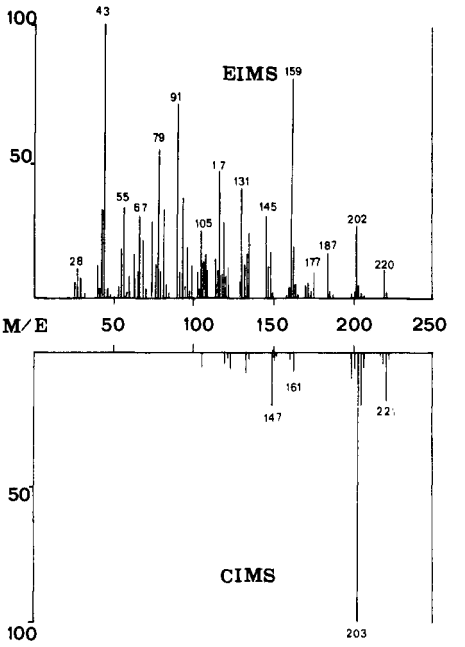

Figure 2. Electron impact (EI) and chemical ionization (CI) mass spectra of the antifeeding compound.

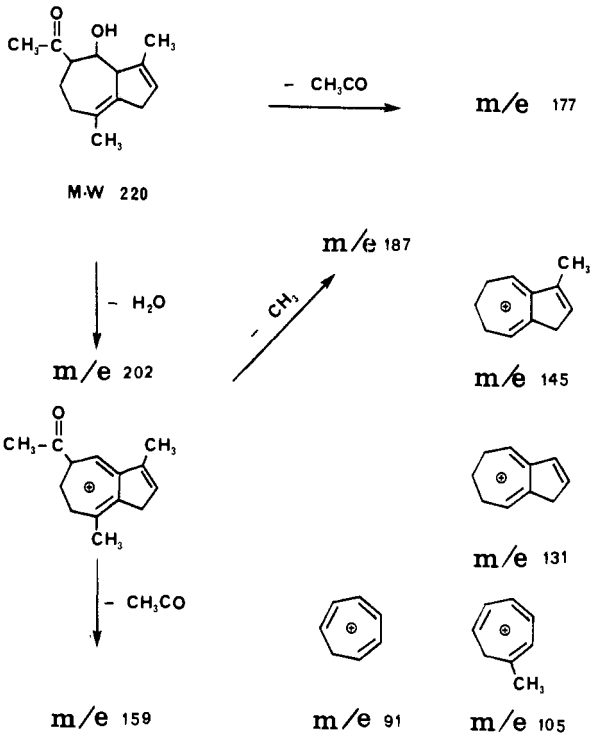

Figure 3. Structure of the antifeeding compound and its major MS ion fragments.

Capillary gas chromatography/mass spectrometry of the crude steam distillate revealed the presence of at least 30 components (Figure 1). However, the purified column fraction with highest antifeeding activity showed one major GC peak (retention time of 11.24 min). The mass spectrum of the active compound is shown in Figure 2. MS library search of the spectrum suggested the structure 1-(1,3,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)ethanone. The suggested structure and the possible structures of the major MS ions are shown in Figure 3. This structure was confirmed by infrared and by proton NMR spectroscopy [IR (liquid film -OH) 3460, (-$COCH_3$)

1705, (C=C) 1640 cm^{-1}; ^1H NMR (in $CDCl_3$) one vinylic proton at 5.55, one methyl group at 1.98 for the aceto group, two methyl groups at 1.35, and other protons signals that were difficult to be assigned due to the overlapping and complex coupling pattern at the used field strength]. The absolute configuration of the suggested structure could not be determined with the available data.

Several compounds containing the azulene nuleus and possessing biological activities, i.e., antifungal, antibiotic, antineoplastic, and ichthyotoxic, have been isolated from algae (Howard and Fenical, 1981; Sun et al., 1981; Moore, 1979), from soft corals (Izac et al., 1981; Fusetani et al., 1981; Kashman et al., 1982) and from desert plants (Shafizadeh and Bhadane, 1972; Burnett and Jones, 1978; Tressl et al. 1983).

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Registry No. 1-(1,3,4,5,6,7-Hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)ethanone, 55683-15-3.

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A Study of the Absorption, Excretion, Metabolism, and Residues in Tissues in Rats Fed Carbon-14-Labeled Sulfamethazine

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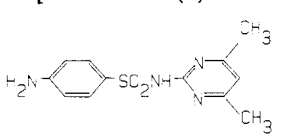
Sprague-Dawley male and female rats received medicated feeds containing 10, 40, 160, 640, 1280, and 2560 ppm of [*phenyl*-U-^{14}C]sulfamethazine for 7 days. Urinary radioactivity accounted for 51–68% of the dose in females and 31–45% in males. Sulfamethazine and N^4-acetylsulfamethazine accounted for 76–84% of the radioactivity in females and 22–63% in males. The N^4-glucose conjugate of sulfamethazine and two other sulfamethazine conjugates, on average, accounted for 73% of the urinary metabolites in males at drug levels below 640 ppm. At drug levels above 640 ppm, the concentration of these conjugates decreased in urine to suggest that there was a saturable process(es) in the male. Females also exhibited consistently higher ^{14}C residues in tissues and blood to further indicate there were apparent sex-related differences in the metabolism of sulfamethazine in rats. Sulfamethazine and N^4-acetylsulfamethazine were identified in the liver. No strain-related differences were noted in sulfamethazine metabolism in a study with Fischer-344 male rats.

Sulfamethazine [4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide] is widely used in combination with antibiotics as feed supplements to promote growth and prevent disease in swine and cattle. Current regulations allow a tolerance of 100 ppb in uncooked edible tissues. Within the past few years sulfamethazine has come under close scrutiny because of the high rate of violative residues above the established tolerance (Trabosh, 1978). The major causes of the violative residue problem have been attributed to the cross contamination of nonmedicated feeds, improper drug withdrawal procedures, and the coprophagic nature of swine.



SULFAMETHAZINE

A request to increase the tolerance levels of sulfamethazine residues in feed and swine tissue so that the residue problem would be eased was reviewed by the Food and Drug Administration, and they decided that until new information became available that would allow modification of the established tolerances for tissues and feed, the current regulations would be retained. Accordingly, they initiated life-time feeding studies in two rodent species at

Metabolism Laboratories, Agricultural Research Division, American Cyanamid Company, Princeton, New Jersey 08540.

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