concentration in "Semmes" leaves, and (c) the higher rate of polar product formation in "Coker 338".

The amount of radioactivity in the residue fraction was equal in the leaves of the two cultivars (Table II). In stems, the level of residue radioactivity was significantly higher in "Coker". However, the restriction of radioactivity in stems to the vascular strands, which have low levels of chlorophyll and thus photosynthesis, argues against residue incorporation as a cause of "Coker" tolerance. Furthermore, the toxiphoric group on metribuzin, the primary amine (Draber and Buchel, 1969), is probably conjugated or sterically hindered before incorporation into the residue. Residue incorporation was higher in roots of "Coker". However, roots do not carry out photosynthesis, thus eliminating this as a cause of differential tolerance. For these reasons incorporation into the residue is not thought to explain differential tolerance.

The restriction of radioactivity to the vascular tissue is interpreted as being caused by metribuzin metabolism to a product that will not penetrate through a membrane. Thus "Coker" traps the products in the veins. "Semmes" metabolizes metribuzin at a much lower rate; thus metribuzin penetrates through the membranes and inhibits photosynthesis.

ACKNOWLEDGMENT

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Maillard Polymers Derived from D-Glucose, D-Fructose, 5-(Hydroxymethyl)-2-furaldehyde, and Glycine and Methionine

Milton S. Feather[*1] and Deanna Nelson

Water soluble, nondialyzable Maillard polymers having molecular weights in excess of 16 000 were prepared from 5-(hydroxymethyl)-2-furaldehyde (HMF), D-glucose, or D-fructose and glycine. Similar polymers were prepared from the latter sugars and methionine. In all cases, the polymers showed no absorption maxima in the 220–320-nm range. For the preparations derived from the latter two sugars, elemental analyses were similar and indicated a nitrogen content of over 6%. Elemental analyses suggest that the polymer is composed of 1 mol of sugar and 1 mol of glycine minus about 3 mol of water. Studies using 90 atom % enriched D-glucose-1-^{13}C, glycine-1-^{13}C, and glycine-2-^{13}C as precursors in the reactions and ^{13}C NMR as a probe show that both carbon atoms of glycine are incorported into the polymer and that C-1 of D-glucose appears as a substituted methyl group. The NMR data further suggest that the main monomeric (dialyzable) products are unreacted sugar or amino acid and 1-deoxy-1-(N-glycino)-D-fructose derivatives (Amadori compounds).

The reaction of reducing sugars with amino acids or protein to produce brown polymers was orginally described by Maillard. Since that time, this reaction has been the subject of numerous studies, and more recently several symposia have been held on the subject (Eriksson, 1982; Waller and Feather, 1983). Although monomeric food flavor and aroma compounds, reductones, and ultraviolet-absorbing compounds are known to be produced in the reaction, the origin and constitution of the polymers are not, at present, well understood. Barbetti and Chiappini (1976a,b) have studied some model systems recently, as have Ledl and co-workers (Ledl, 1982a,b; Ledl and Severin, 1982) and Velisek and Davidek (1976a,b). More recently (Imasato et al., 1981; Bobbio et al., 1981), reports have appeared that describe the preparation and fractionation of melanordins from D-glucose, D-fructose, D-xylose, and glycine. Analytcal data, including elemental analyses and IR and acetylation data were reported.

A knowledge of the chemical constitution of Maillard polymers is desirable, since they are known to contribute to the discoloration of many foods and may have an effect on the digestibility and mineral binding properties of processed foods.

The purpose of this report is to describe the isolation of Maillard polymers derived from D-glucose, D-fructose, or 5-(hydroxymethyl)-2-furaldehyde and glycine and to report analytical data (UV, NMR, and elemental analyses)

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that relates to their composition and structure. Some preliminary information on polymers derived from sugars and methionine is also reported.

EXPERIMENTAL SECTION

Materials and Methods. Carbon-13-enriched amino acids and sugars were obtained from Merck Sharp & Dome. Ultraviolet spectra were obtained on a Cary Model 219 spectrophotometer. NMR data were collected on a JEOL FX 270 superconducting instrument (located at Northwestern University) and a Nicholet 300-MHz superconducting instrument (located at the University of Missouri—Columbia). Samples were run in deuterium oxide solutions with dioxane as an internal reference. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, IL.

Polymer Yield Determinations. In a typical experiment, 200 mL of phthalate buffer (pH 3.5), 0.2 M with respect to carbohydrate, and amino acid were heated to reflux in a 250-mL round-bottom flask equipped with a condenser. The time required to reach reflux was 0.5 h, and this was arbitrarily designated as "zero" time. Over an 8-h period, 10-mL aliquots were removed and placed in 10 mm diameter dialysis bags. The samples were dialyzed against running tap water for 48 h and then against 4–2-L changes of distilled water over a 24-h period. The contents of the dialysis tubes were transferred to 6-cm evaporating dishes and evaporated to dryness first at atmospheric pressure and then in a vacuum desiccator over Drierite to constant weight. Polymer yields were determined gravimetrically. The dried polymers were readily soluble in water and were reconstituted and suitably diluted for ultraviolet spectral determinations.

Polymer Preparation. In a typical experiment, 36 g of D-glucose (0.2 mol) and 15 g of glycine (0.2 mol) were added to 200 mL of phthalate buffer (pH 3.5), and the solution was refluxed for 8 h. After being cooled, the solution was placed in 27-mm dialysis tubing (nominal molecular weight cutoff = 16 500), dialyzed against running tap water for 72 h, and then dialyzed against 4–5-L changes of distilled water for 24 h. This solution was filtered to remove traces of particulate matter and then freeze-dried. The yield was 1.26 g, and the sample was completely soluble in distilled water. All other large-scale reactions used as identical procedure.

Carbon-13-Enriched Preparations. In a typical experiment, 200 mg of D-glucose-1-^{13}C (90 atom %) and 84 mg of ordinary glycine were dissolved in 1.12 mL of phthalate buffer (pH 3.5). This solution was sealed in a glass tube and immersed in a boiling water bath for 8 h. After being cooled, the solution was placed in a 10-mm dialysis bag and dialyzed against 1 L of distilled water for 24 h. The external solution was evaporated to dryness at atmospheric pressure and the sample used in NMR experiments to examine the dialyzable reaction components. The polymeric solution (inside the dialysis bag) was further dialyzed against 10 1-L portions of distilled water for an additional 10 days with one change of water per day. It was then removed from the bag, evaporated to dryness to atmospheric pressure, and used in NMR experiments. All other isotope enriched samples were processed in the same manner.

RESULTS AND DISCUSSION

In acidic solutions, it is generally conceded (Scheme I) that the initial reaction between a reducing sugar and an amino acid involve the condensation of sugar (1) with the amino group to give a glycosylamine (2), which rearranges to an Amadori compound (3). Amadori compounds are
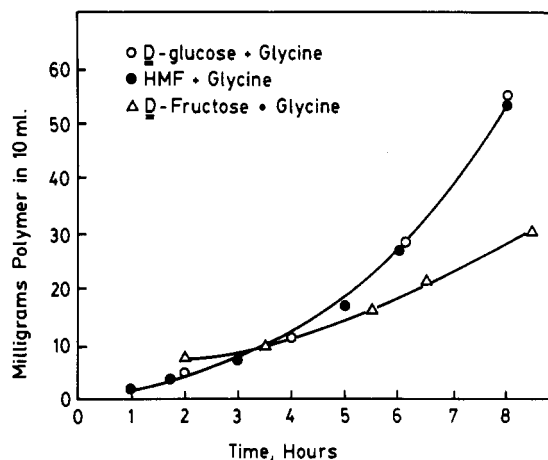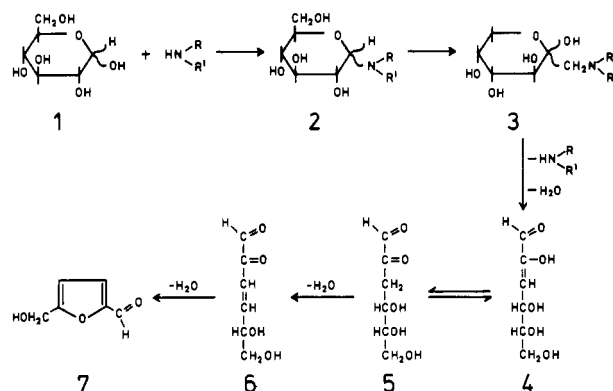
Scheme I

Figure 1. Yields of nondialyzable polymers as a function of time using glycine as the amino acid.
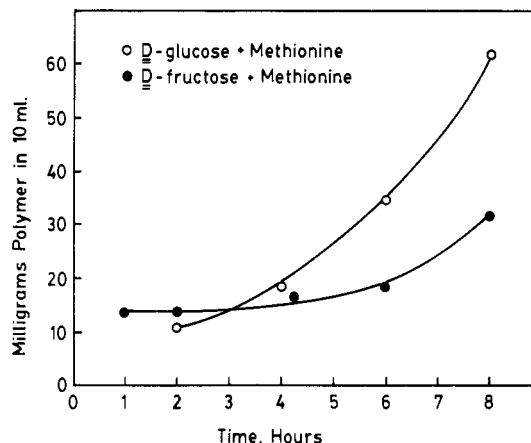
Figure 2. Yields of nondialyzable polymers as a function of time methionine as the amino acid.

known to readily degrade (with loss of the amino substituent) to give the enolic form (4) of a 3-deoxyosone (5), which then further dehydrates to 6 and then to 5-(hydroxymethyl)-2-furaldehyde (HMF, 7). During this degradation reaction, Maillard polymers are formed as well. Little is known about the role or importance of intermediates 4–6 or of 7 in the polymerization reaction that leads to Maillard polymer formation or of the role of the released amino acid in such a reaction.

The studies described here involved reactions of equimolar amounts of carbohydrate (D-glucose, D-fructose, and 7) and glycine (0.2 M) in solutions buffered at pH 3.5 at 100 °C (reflux). At these conditions, the solutions rapidly darkened, but negligible material precipitated at reaction times up to 8 h.
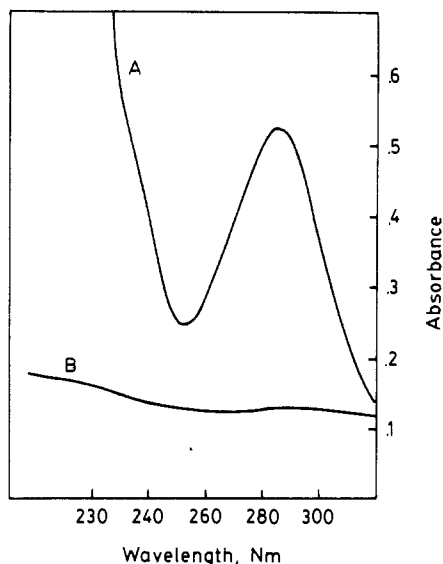
Figure 3. Ultraviolet absorption spectra for (A) a reaction solution of D-glucose and glycine initially 0.2 M for each reactant (diluted 1:2000 at 8-h reaction time) and (B) a Maillard polymer produced at the same connditions.

Table I. Elemental Analyses of Some Maillard Polymers Prepared from Glycine and Various Reactants

reactant	% C	% H	% N
D-glucose	51.20	5.28	6.24
D-fructose	50.48	4.87	6.87
HMF (7)	47.63	3.72	3.53

In all cases, water-soluble, nondialyzable polymeric material was produced. Figures 1 and 2 show plots of polymer yields vs. time for the reactions involving glycine and methionine. Reducing the ratio of carbohydrate:amino acid to 1:0.1 substantially reduced the yield of pclymer (and color development as evidenced by visual inspection).

It is also noteworthy that (by visual observation) reactions involving D-fructose and glycine or methionine darkened much more rapidly than those involving D-glucose, even though the overall yield of polymer was substantially lower. This may be due to the fact that D-fructose undergoes acid-catalyzed degradation more readily than aldoses and that, at the conditions used (initial pH 3.5), this side reaction was more prominent.

Reaction solutions rapidly gave rise to ultraviolet-absorbing compounds. For all systems studied, a broad absorption band was observed at about 285 nm (Figure 3). The polymer, after extensive dialysis and isolation (by lyophilization), showed little absorption (Figure 3). The absence of spectral absorption is surprising, since 7 itself (which is produced in the reaction and would be predicted to be a reactant) shows strong absorption bands at 230 and 280 nm. For it to participate, either ring opening must occur or other molecules must add to the double bond system, destroying the conjugation. Also noteworthy is the fact that Barbetti and Chiappini (1976a) have reported that Maillard polymers derived from D-glucose and glycine show substantial ultraviolet absorption and that they contain an intact furan ring. In contrast, Olsson et al. (1982) recently reported that similar polymers show no ultraviolet absorption and that the ^{13}C NMR spectra of these materials resembles that of an Amadori compound derived from D-glucose and glycine [1-deoxy-1-(N-glycino)-D-fructose].

Elemental analyses (carbon, hydrogen, and nitrogen) for the polymers (Table I) are similar and suggest that the amino acid is incorporated into the material. For the
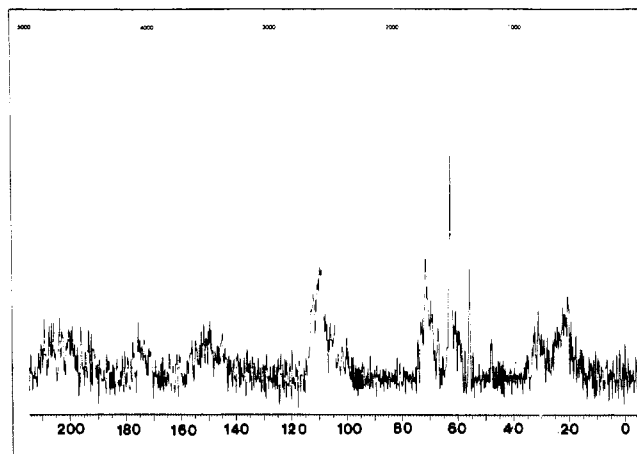

Figure 4. Natural abundance ^{13}C NMR spectrum of a Maillard polymer derived from D-glucose and glycine.

polymers derived from D-glucose or D-fructose and glycine, calculations suggest that the polymer is composed of sugar and amino acid minus about 3 mol of water.

Further evidence that the amino acid is incorporated into the polymer was found from the analysis of a polymer prepared from D-glucose and methionine. This preparation had the following elemental analysis: C, 42.24; H, 4.35; N, 3.30; S, 4.66. That both nitrogen and sulfur are incorporated suggests that participation of intact amino acid in the reaction.

By qualitative observation, the polymers were more soluble in tap water than in distilled water. Furthermore, the polymers appeared to irreversibly bind metal ions, which could not be removed by dialysis against distilled water. This observation stems from the fact that polymers isolated by dialysis against tap water gave no ^{13}C NMR signals (consistent with the presence of bound paramagnetic ions), while those processed in ion-free water did. The binding of metal ions, particularly iron, to melanoidins is not a new observation. A recent report (Hashiba et al., 1982) has discussed the catalytic effects of certain metals on browning.

Carbon-13 nuclear magnetic resonance was used to further examine the constitution of the polymers. A natural abundance ^{13}C NMR spectrum of the polymer (Figure 4) prepared from D-glucose and glycine was similar to that reported by Olsson et al. (1982). In addition, the spectrum collected in this study showed a series of signals at 20–35 ppm as well as at 160–210 ppm. While it is not possible to make definitive assignments on such a complex system, the former signals could represent substituted methyl or methylene carbons, while the latter suggest the presence of some aromaticity. Olsson et al. (1982) point out that the NMR spectrum is similar to that of an analogous Amadori compound. Clearly, the polymer is not composed of Amadori compounds linked to one another, since the elemental analyses show that about 3 mol of water is eliminated during the reaction. It seems more probable that dehydrated, sugar-derived intermediates such as 4–7 are involved and that these react in an as-yet-unexplained manner with amino acid to produce the Maillard polymer. It is also noteworthy that Benzing-Purdie and Ripmeester (1983) have prepared melanoidins from D-glucose and D-xylose and glycine and have compared the NMR spectra of these preparations with that of soil humic material. They have concluded that such materials are highly aliphatic and, in addition, contain aromatic, carbonyl, and carboxyl carbons. In additional experiments, reactions were run using 90 atom % D-glucose-1-^{13}C, glycine-1-^{13}C, and glycine-2-^{13}C as starting

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A

B

200 180 160 140 120 100 80 60 40 20 0

A

200 180 160 140 120 100 80 60 40 20 0

B

200 180 160 140 120 100 80 60 40 20 0

Figure 6. ^{13}C NMR spectra of Maillard polymers prepared from D-fructose and glycine-1-^{13}C (90 atom %) (A) and from D-fructose and glycine-2-^{13}C (90 atom %) (B).

200 180 160 140 120 100 80 60 40 20 0

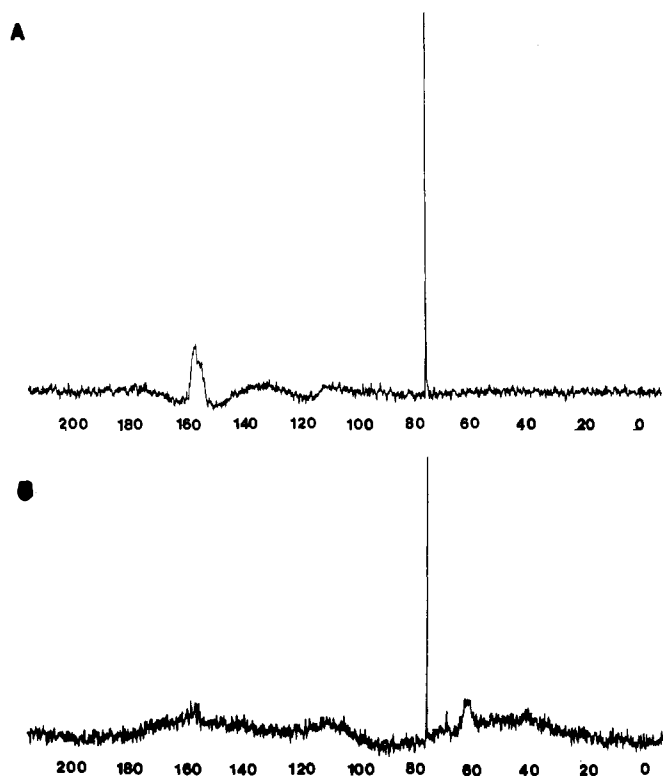**Figure 5.** ^{13}C NMR spectra of Maillard polymers prepared from D-glucose and glycine-1-^{13}C (90 atom %) (A), and from D-glucose and glycine-2-^{13}C (90 atom %) (B). The sharp signal at 67.2 ppm represents dioxane, used as an internal standard.

materials and the dialyzable and nondialyzable fractions isolated. For the experiment using glycine-1-^{13}C, the dialyzable material showed three major signals at 173.0, 172.1, and 170.8 ppm. The signal at 173.2 is assigned to C-1 of glycine. The reported chemical shift is 173.2 ppm (Stothers, 1972). The latter signals are assigned to an Amadori product derived from D-glucose and glycine-1-^{13}C. Kraska et al. (1975) reported the carbonyl reasonance for this Amadori compound to be at 172.1 ppm. Funcke and Klemer (1976) have reported quantitative ^{13}C data on a series of Amadori compounds and have shown that two major forms exist in solution, namely, the β-pyranose and the α-furanose forms. The signals at 172.1 and 170.8 ppm are consistent with their observation. On the basis of signal intensity, the largest signal (172.1 ppm) could be assigned to the more abundant β-pyranose structure and that at 170.8 ppm to the α-furanose.

The dialyzable material derived from the glycine-2-^{13}C experiment showed a signal at 44.4 ppm (C-2 of unreacted glycine) (Stothers, 1972) and two additional ones at 49.7 and 40.8 ppm that are assigned to 1-deoxy-1-(N-glycino-2-^{13}C)-D fructose derivatives. For the experiment using D-glucose-1-^{13}C as the starting material, signals were observed at 95.9 and 92.0 ppm corresponding to the β- and α-pyranose anomers of unreacted sugar. Three additional upfield signals at 53.2, 52.3, and 51.2 ppm were also present. These signals have the expected chemical shifts for C-1 of the sugar when it has been converted to an Amadori compound. Funcke and Klemer (1976) report values ranging from 51.5 to 67.1 ppm for similar compounds.

Although the reaction mixtures appear complex and dark in color, it appears that the major dialzable products of the reaction are unreacted sugar, amino acid, and Amadori compounds derived from them.

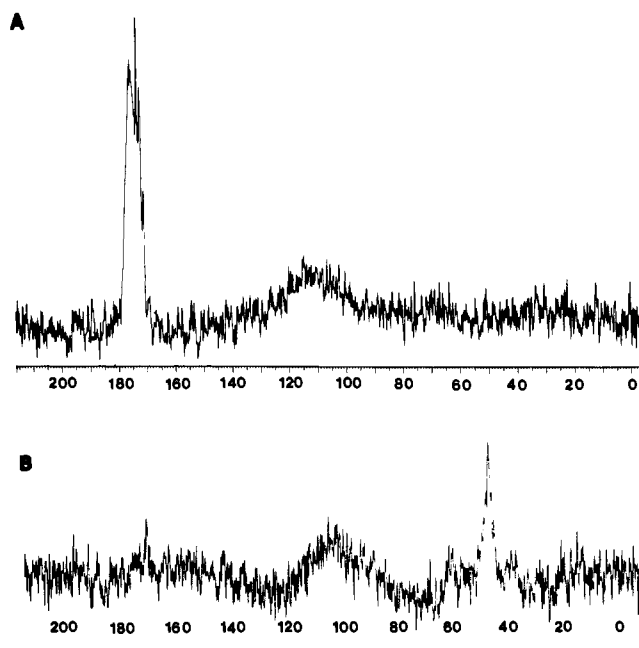The NMR spectra of the nondialyzable polymers showed a broad signal centered at 155–160 ppm for the material

prepared from glycine-1-^{13}C (Figure 5). This is in the expected region for a carbonyl carbon. This is interesting, since in his original paper Maillard (1916) suggested that this carbon is lost as carbon dioxide during polymer formation. This has been experimentally verified since that time (Wolfrom et al., 1953). It would appear (based on these data) that some of C-1 of glycine is released as CO_2 and some is incorporated into the polymer. The material prepared from glycine-2-^{13}C showed a series of signals at 60 ppm (Figure 5), consistent with substituted methyl groups, while the material made with D-glucose-1-^{13}C showed signals in the region 41.4–49.7 ppm, again in the region for a substituted methyl group. It appears that both carbon atoms of glycine are incorporated into the polymer and that C-1 of D-glycose is no longer a carbonyl carbon when the reaction is complete. It is also noteworthy that Kraska et al. (1975) reported that for 1-deoxy-1-(N-glycino)-D-fructose, the resonances for C-1 (sugar) appear at 54.3 ppm and for the amino acid portion at 172.1 ppm (carboxyl) and 50.8 ppm (C-2). These assignments are similar to those found for the actual polymer and suggest that it might well result from the dehydration–polymerization of an Amadori compound as has been suggested by Olsson et al. (1982).

Some additional studies were performed using D-fructose and glycine-1- and -2-^{13}C. Similar NMR data were collected. The dialyzable portion from the glycine-1-^{13}C showed signals at 175.6, 173.6, and 172.1 ppm, and the preparation starting with glycine-2-^{13}C showed signals at 43.4, 42.7, and 42.2 ppm. The polymeric material containing glycine-1-^{13}C gave signals centered 175 ppm and that containing glycine-2-^{13}C (Figure 6) showed signals centered at 45 ppm. The NMR data suggest that some structural differences exist between the D-glucose- and D-fructose-derived polymers. This is indicated by the chemical shifts shown by the glycine-1- and -2-^{13}C-enriched materials. The signals for the glycine-1-^{13}C-labeled polymers is shifted downfield by about 20 ppm for the D-fructose-derived material (relative to the D-glucose polymer) and the glycine-2-^{13}C signals are shifted upfield by about 15 ppm (relative to the D-glucose polymer). In all other respects (UV spectra and elemental analysis), the

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*

Mahmoud Abbas Saleh,* Nadia M. Abdel-Moein, and Nagy A. Ibrahim[1]

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

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identified to be 1-(1,3,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)ethanone.

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;