prolonged heating at temperature close to the denaturation temperature, oat globulin (in dilute solutions) seems to retain considerable native structure since the urea-perturbed protein exhibited further denaturation as indicated by more pronounced spectral changes. There was a segregation of native and denatured proteins into soluble and insoluble fractions, respectively, when oat globulin was heated at higher (1%) concentration. Spectral data and surface hydrophobicity measurements suggest that insoluble aggregates were formed from extensively denatured proteins. This is consistent with the generally accepted view that aggregation is preceded by denaturation, following the scheme $N \rightleftharpoons D \rightarrow A$, where N denotes native protein, D denatured protein, and A the aggregate (Ferry, 1948).

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Stability of Gliadin-Encapsulated Unsaturated Fatty Acids against Autoxidation

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The antioxidant effects at various A_w of spray-dried powders prepared from alcoholic solutions of gliadin, linoleic acid, and palmitic acid were compared against powders prepared by simple mixing of these components in the same portions and against gelatin or starch powders substituted for gliadin. The effectiveness of gliadin at low or moderate A_w in the simple mixture that was not as good as at high A_w was greatly improved via the process of spray-drying. This process caused a significant loss in the extraction efficiency of fatty acids by hexane but did not influence the in vitro digestion of gliadin. The outer surface of the spray-dried gliadin particles containing the lipid was characterized by the disappearance of deep dents otherwise observed by SEM. These findings imply that the lipid droplets must have been embedded in the gliadin matrix so as to avoid oxygen attack.

In the preceding paper (Iwami et al., 1987), we demonstrated that gliadin, a component of wheat gluten, functions as the best antioxidant among available food proteins against linoleate peroxidation in powder model systems and that its effectiveness continues longer at high A_w. Gliadin was never superior to other proteins in radical and sulfhydryl reactions. Since this protein is so abundant in glutamine and proline as to scarcely dissolve in water (Pomeranz, 1968), its physicochemical properties are of interest in connection with the antioxidant ability.

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Microencapsulation is a technique by which oily substances are entrapped in wall materials and are protected from injurious circumstances such as oxygen, moisture, and light (Ono, 1980). Spray- or freeze-dried powder particles are considered to be of the microcapsule kind. In this connection, Gejl-Hansen and Flink (1977) described that when linoleic acid is emulsified in maltodextrin-containing aqueous solution in the presence of detergents and is entrapped in the dextrin matrix by freeze-drying, it becomes unsusceptible to oxidation. On the other hand, Ono and Aoyama (1979) reported that rice bran oil embedded in the granules consisting of corn syrup solid and pork polypeptone by vacuum drying undergoes little oxidation on exposure to air at a high temperature for a few weeks. In many cases, spray-drying is preferably employed to produce microcapsules than freeze-drying for the sake of convenience and manufacturing cost (Brown et al., 1973; King 1973). Dry milk and egg powders are such practical examples, which are relatively stable to oxidative deterioration during a long-term storage.

Amphiphilic proteins are not only soluble in water but also miscible with lipids, so that it is appropriate to utilize them as wall materials for encapsulation. In general, however, the presence of detergents besides proteins is required to make a stable emulsion of oil-in-water prior to spray- or freeze-drying. Gliadin is an aqueous alcohol-soluble protein. Unsaturated fatty acids also dissolve in the same solution. These characteristics are favorable for preparing fine proteinous microcapsules containing unsaturated fatty acids by spray-drying.

This investigation was designed to ascertain whether or not the preservability of unsaturated fatty acid could be all the more improved by encapsulation with gliadin.

MATERIALS AND METHODS

Materials. Wheat gluten was purchased from Nakarai Chemicals Ltd., Kyoto (manufactured in spring 1984). The protein (450 g) was suspended in 10 volumes of 70% ethanol with stirring for 1 day at 4 °C, and the supernatant fluid after centrifugation at 8000 rpm for 20 min was spray-dried in order to obtain gliadin as impalpable powders. The conditions for spray-drying are follows: apparatus, Yamato-Pulvis Model GA-21; inlet temperature, 140 °C; outlet temperature, 80 °C; flow rate of dry air, 0.6 m^3/min; flow rate of spray air, 13.3 L/min. Linoleic and palmitic acids were also products of Nakarai Chemicals Ltd. Other chemicals, of analytical grade, were obtained from commercial sources and used without further purification.

Preparation of Samples. The gliadin powders (48 g) were once again dissolved in 2 L of 70% ethanol, followed by addition of linoleic acid (8.4 g) and palmitic acid (3.6 g). The ethanolic solution was spray-dried under the same conditions as above. With respect to simple mixing, 100 mL of hexane solution containing a 10% (w/w) linoleate–palmitate (7:3) mixture was dropwise added to 40 g of gliadin or gelatin or α-corn starch with stirring, and the solvent was thoroughly evaporated in a stream of nitrogen. The samples thus obtained were stored at −20 °C in a nitrogen-filled vessel until use.

Antioxidation Tests. Spray-dried and simply mixed samples were individually divided in small portions (approximately 100 mg/sample) into three groups, which were allowed to stand for desired periods at 40 °C in humidity-controlled desiccators with various concentrations of sulfuric acid (55%, 40%, and 25% by weight for A_w = 0.30, 0.55, and 0.85, respectively) (Troller and Christian, 1978). The respective desiccators were flushed with oxygen after every sampling for peroxide measurements and gas chromatographic analyses. The peroxide value was estimated by a modification of the ferric thiocyanate method (Mitsuda et al., 1966) and expressed as the average of triplicate determinations within the experimental errors. Gas chromatographic analyses were carried out in the same manner as previously described (Iwami et al., 1987). The ratio of linoleic acid to an internal standard "palmitic acid" remaining in the samples, i.e. the L/P ratio, was obtained by calculation from their corresponding peak areas on the gas chromatographic chart.

Colorimetric Determination of Fatty Acid. Twenty milligrams of the freshly prepared samples was extracted by vigorous stirring for a short time with 4 mL of hexane in a Vortex mixer and immediately filtered through a Whatman 1 PS filter paper. The free fatty acid content in the filtrate was estimated according to a modification of the method described by Falkot et al. (1973). Namely, a 0.5 mL of the extract was dissolved in 4.5 mL of a chloroform–heptane (1:1) mixture, to which was added 2 mL of a Cu–TEA reagent containing 0.05 M $Cu(NO_3)_2$ and 33% NaCl in 0.1 M triethanolamine buffer of pH 8.1. The mixture was vigorously stirred in a glass centritube with a screw cap and separated into two layers by centrifugation at 3000 rpm for 15 min. A 100-μL portion of the upper layer was withdrawn and filled up to 3 mL with 99% ethanol. The alcoholic solution was further mixed with 0.5 mL of 0.4% 1,5-diphenylcarbazide in ethanol, and the absorbance at 550 nm was measured exactly after 15 min. These operations were repeated three times to obtain more reliable data.

Scanning Electron Microscopy. Spray-dried gliadin powders with or without fatty acids inside the particles were coated with carbon by the use of a JEOL JEE-4B vacuum evaporator, and then SEM pictures were made on a JEOL JXA-840 scanning microanalyzer.

In Vitro Digestibility. A 300-mg portion of the gliadin-based sample after 6 weeks of storage was suspended in 30 mL of 0.03 N HCl, and to the suspension was added a $^1/_{50}$th amount of porcine pepsin (Sigma Chemical Co.) with stirring. After incubation at 37 °C overnight, pepsin was inactivated by adjustment to pH 8 with NaOH and proteolysis was again initiated by addition of porcine pancreatin (Sigma Chemical Co.) at the same level as pepsin digestion. At stated intervals while digestion by these enzymes proceeds, the reaction mixture was taken out in each 1 mL and deproteinized by adding 2 mL of 15% TCA. The degree of digestion as a function of time was expressed in absorbance at 280 nm corresponding to the amino acids and peptides in the TCA-soluble fractions.

RESULTS AND DISCUSSION

Figure 1 depicts the time course of hydroperoxide formation during storage at 40 °C with respect to spray-dried and simply mixed samples that were in principle composed of gliadin, linoleic acid, and palmitic acid in the 40:7:3 ratio, except that gliadin was replaced by gelatin or starch in the simple mixing system. The peroxide value in the latter mixtures was much elevated on the third day after the beginning of experiment at low A_w. Similar rises in the peroxide values were observed for all the simple mixtures at moderate A_w as well. With respect to the spray-dried powders containing fatty acids, however, their peroxide value reached a maximum a few weeks later at A_w = 0.30 but remained at a constant, low value throughout the experimental period of 6 weeks at A_w = 0.55. At high A_w, the gliadin-based samples were highly resistant to oxidative deterioration irrespective of spray-drying or simple mixing, although linoleic acid in the gelatin- or starch-based sample rapidly oxidized to form hydroperoxides at high levels.

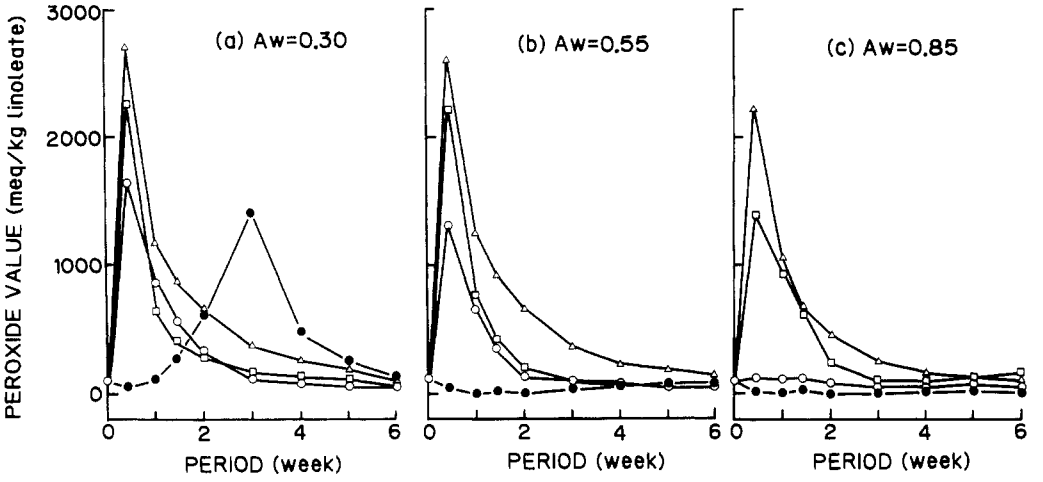

Figure 1. Lipid peroxidation in powder model systems during storage at (a) low, (b) moderate, and (c) high A_w. Powder model systems that had been prepared by spray-drying (gliadin based, ●) and by simple mixing (gliadin based, ○; gelatin based, □; starch based, △) were stored at 40 °C in humidity-controlled desiccators except for time of sampling.

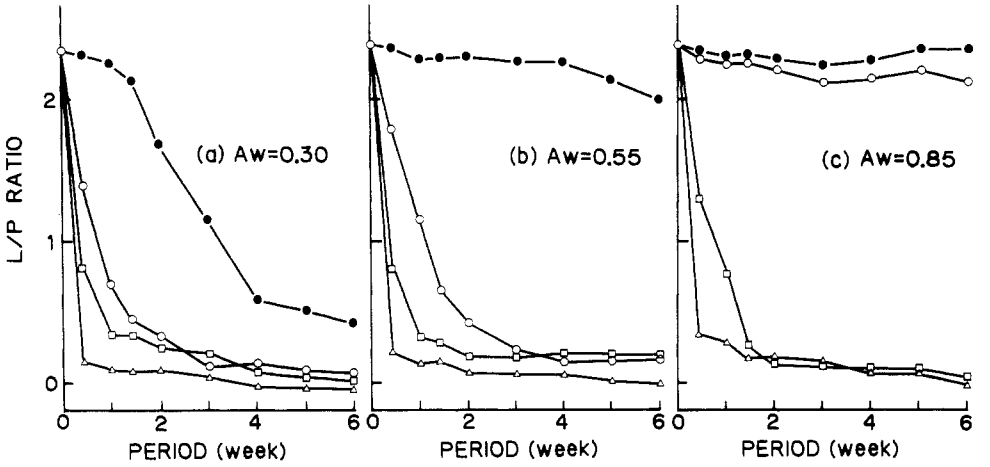

Figure 2. Oxidation-caused disappearance of linoleic acid in powder model systems during storage at (a) low, (b) moderate, and (c) high A_w. Conditions for sample preparation and storage are the same as in Figure 1. The degree of linoleate disappearance was interpreted in terms of the ratio of linoleate to an internal standard "palmitate".

The antioxidant effect was also confirmed by gas chromatographic determination of linoleic acid remaining intact in the respective samples. The results of gas chromatographic analyses are represented in terms of the ratio of linoleic to palmitic acid (L/P ratio) in Figure 2. Alterations in the L/P ratio were faithfully reflected in the changes of peroxide values given in Figure 1. Namely, the samples in which the peroxide value was maintained at a marginal level gave a constant or sparingly variable L/P ratio. As the peroxide value was elevated, the L/P ratio decreased rapidly; the tendency was pronounced with respect to both the starch- and gelatin-based samples even under different conditions in humidity. On the other hand, the degree of linoleate peroxidation in the gliadin-based sample was influenced by their preparation procedure and humidity during storage. Gliadin that was simply mixed with linoleic acid did not exhibit a high antioxidant activity at low and moderate A_w, whereas spray-drying together with the fatty acids caused a great improvement in protection from oxidative deterioration. As shown in Figure 2b,c, the L/P ratio of the spray-dried sample very slowly decreased at A_w = 0.55 but hardly varied at A_w = 0.85 throughout the experimental period.

Figure 3 compares the efficiency of fatty acid extraction by hexane from the spray-dried and simply mixed gliadin samples in order to account for the difference between the two samples in resistance to oxidation. More than 90% of the total fatty acids was extracted from the simply

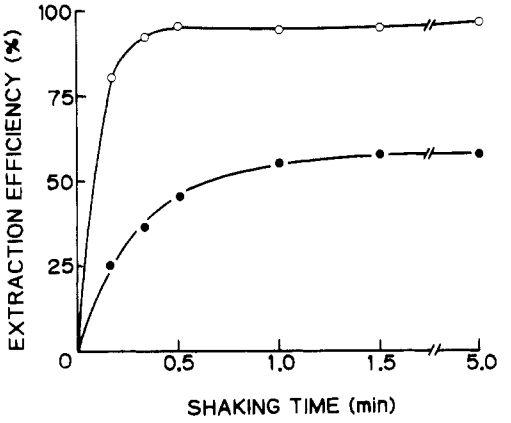

Figure 3. Extraction efficiency of fatty acids with hexane from gliadin-based samples that were prepared by spray-drying (●) and by simple mixing (○) together with linoleate–palmitate (7:3) mixture. The procedures for fatty acid extraction and determination were in detail described in Materials and Methods.

mixed sample by vigorous stirring for a mere 20 s, but more prolonged stirring did not cause a further increase in recovery. In contrast, the extraction efficiency of fatty acids from the spray-dried sample increased with stirring times and reached a plateau in several minutes to the extent not exeeding 60%. Figure 4 shows typical SEM photographs of gliadin powders spray-dried in the absence and presence

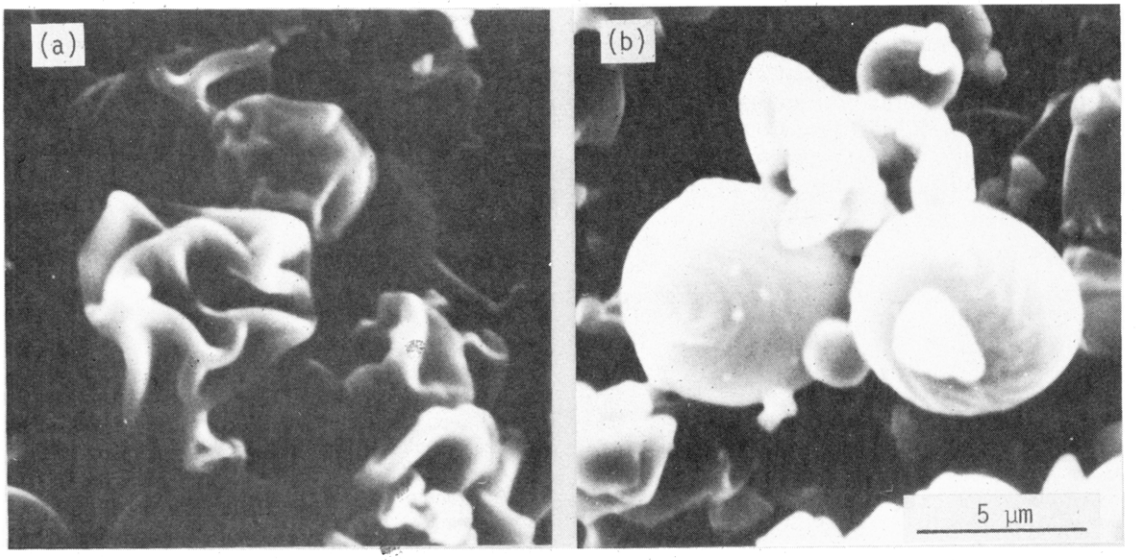

Figure 4. Typical SEM photographs of spray-dried gliadin particles prepared in the absence (a) and presence (b) of 20% (w/w) linoleate–palmitate mixture.

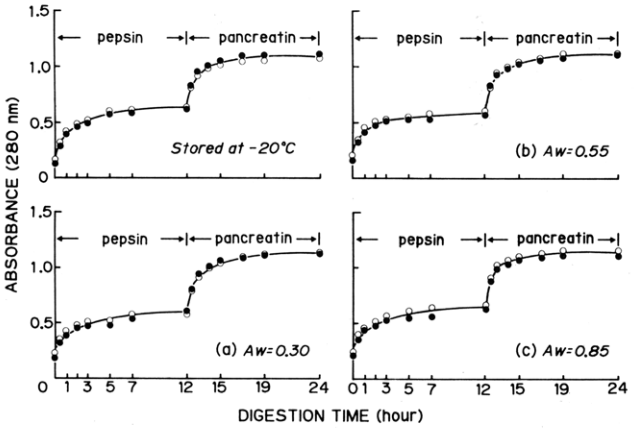

Figure 5. Effect of storage at various water activities on in vitro digestibility of gliadin-based samples that were spray-dried (●) and simply mixed (○) with linoleate–palmitate mixture. The details of conditions for digestion by pepsin and pancreatin were described in Materials and Methods.

of fatty acids. The gliadin particles containing fatty acids were found spherical in shape but variant in size, while some creases or dents were seen on the surface of those not containing fatty acids. This observation and the decrease in the extraction efficiency of fatty acids by spray-drying leads to the assumption that the majority of fatty acids would be embedded in the gliadin matrix relative to those in the simply mixed sample where their adsorption on the surface of gliadin particles with deep dents would predominate.

Gliadin had a continued antioxidant effect at high A_w, but not at low Aw, as mentioned above. Figure 5 shows the results of examination as to whether or not a long-term storage at different A_w affects the digestibility of gliadin in the spray-dried or simply mixed sample. The samples that had been stored at −20 °C in a closed vessel filled up with nitrogen over a period of 6 weeks exhibited change neither in the peroxide value nor in the L/P ratio. No significant difference was observed in digestibility between this and other samples. Similar profiles were obtained with the samples stored for 6 weeks at 40 °C and various A_w. At low A_w, linoleic acid was almost missing in the sample after 6 weeks of storage; nevertheless, gliadin was as effectively digested as at moderate and high A_w. Hence, spray-drying has proven to raise the antioxidant effect of gliadin without impairing its digestibility.

There is little information on the mechanism by which gliadin elicits such a prominent antioxidant effect at high A_w. Circumstantial evidence indicates that an explanation for the mechanism is found, if anything, largely in physical and chemical properties of gliadin. For example, the difference at $A_w = 0.30$ and 0.55 between the simply mixed and spray-dried gliadin samples may be mainly explained by a physical phenomenon. At both A_w, the droplets of unsaturated fatty acid located on the surfaces of dented gliadin powders can be easily attacked by oxygen. Also, those within the spray-dried gliadin particles can be gradually oxidized through cracks and pores at $A_w = 0.30$, while oxygen cannot penetrate at $A_w = 0.55$ because of the possible swelling and fusing of the cracks and pores in analogy with the previous observation of Rosenberg et al. (1985). It seems more likely at $A_w = 0.85$ that a chemical phenomenon is not only involved in the similarity in the simply mixed and spray-dried gliadin samples but also in the difference between these and the gelatin- or starch-based sample. In most food systems, their exposure to high humidity leads to an increase in the rate of lipid peroxidation, probably by raising the metal-catalyzed decomposition of hydroperoxides to produce alkoxy and hydroxyl radicals and/or by elevating the mobility and reactivity of these and other radical intermediates. On the contrary, gliadin in both simply mixed and spray-dried samples elicited a high antioxidant activity under a moist environment. Certain amino acid moieties on this protein would react with lipid peroxy radicals to retard the propagation steps in the antioxidation mechanism. The importance of specific amino acid moieties occurring in the gliadin molecule is supported by the finding that gelatin or starch has no or little antioxidant activity at various A_w. It is however uncertain what amino acid moieties are responsible for potential sites of reaction. In this connection, it is noteworthy that deamidation of glutaminyl residues highly contained in gliadin causes a decisive loss in its antioxidant activity even at high A_w (unpublished data).

Spray-drying is a useful and convenient tool for encapsulation of volatile materials or oily substances, although many microencapsulation techniques have been referred to in recent literature (Chang, 1984; Yapel, 1985; Tomlinson and Burger, 1985). The presence of microcapsules containing lipid droplets in spray-dried gliadin powders can be assessed by SEM photographs representing diverse

spherical particles as well as by scantness of hexane-extractable lipid from the powders that had been put up in a desiccator with silica gel immediately after spray-drying. The microcapsules thus obtained are so resistant to oxidative deterioration during a long-term storage at various A_w that they can be applied to food processing as additives fortified with polyunsaturated lipid. The practical application remains to be further investigated.

ABBREVIATIONS

A_w, water activity; SEM, scanning electron microscopy; TCA, trichloroacetic acid.

Registry No. Linoleic acid, 60-33-3; palmitic acid, 57-10-3; starch, 9005-25-8.

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Pyrazinyl-Substituted 1,3-Cycloalkanediones and Related Compounds: Synthesis and Pesticidal Properties

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2-(2-Pyrazinyl)-1,3-cyclohexanediones with 3-methyl substituents on pyrazine show significant post- and preemergence herbicidal activity, particularly against grass species, and are also active against the adult two-spotted spider mite. While 5- or 6-methyl substituents alone did not impart mite activity, 5-methylpyrazine substitution significantly enhanced activity against grasses. 3,5,6-Trimethyl substitution effected the maximum toxicity against both grass species and adult mites. In the 2-(3,5,6-trimethylpyrazinyl) dione series mite activity appeared to increase with increasing size of the dione C-5 alkyl substituent. No acaricidal or herbicidal activity was observed for 2-(4-pyrimidinyl)-, 2-(3-pyridazinyl)-, or 2-(*N*-methyl-2-imidazolyl)dimedones. Introduction of a 4-methyl group into the pyrimidine ring, however, caused a significant increase in herbicidal activity, particularly against broadleaf species.

2-(2-Pyridinyl)-1,3-cyclohexanediones show marked broad-spectrum herbicidal activity (Manning et al., 1988) without activity on mites or insects, in contrast to appropriately substituted 2-phenyl-1,3-cyclohexanediones and their enol esters, which show selective toxicity to grass species and are also active acaricides, against both adults and eggs (Wheeler, 1980). The two dione classes differ strikingly in their relationships between aryl ring substituents and activity. Whereas the herbicidal 2-pyridinyl diones appear to require the ability to form an internally hydrogen-bonded N---HO— chelate ring, permitted by essential coplanarity of the pyridine ring and the enolic dione C=C (Manning et al., 1988), a phenyl ortho substituent precluding such coplanarity is a general requirement for both herbicidal and acaricidal activity in the 2-phenyl-1,3-cyclodione series (Wheeler, 1980). Thus, it was of interest to synthesize and test 1,3-cyclohexanediones with pyrazine and other diazines attached at the dione 2-position.

METHODOLOGY

Synthesis. Attachment of diazine rings to the 2-position of 1,3-cyclohexanediones was accomplished by initial synthesis of appropriate 6-diazinyl-5-oxohexanoic acid esters via condensation of lithiated methyldiazines with 3-substituted glutaric esters. Cyclization of the 5-oxohexanoate esters gave the diones, all steps analogous to

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