Gas Chromatographic Analysis of Glyoxal and Methylglyoxal Formed from Lipids and Related Compounds upon Ultraviolet Irradiation

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Glyoxal, methylglyoxal (MG), malonaldehyde (MA), acrolein, formaldehyde, acetaldehyde, and propanal formed from squalene, cod liver oil, ethyl esters of fatty acids, and volatile aldehydes upon UV irradiation were derivatized to nitrogen- or sulfur-containing compounds. Derivatives were analyzed by a gas chromatograph equipped with a nitrogen–phosphorus detector or a flame photometric detector. Maximum amounts of glyoxal (9.6 nmol/mg) and MG (14 nmol/mg) formed from squalene after 10 h of UV irradiation. Cod liver oil also produced glyoxal (27 nmol/mg) and MG (5.7 nmol/mg). Glyoxal was formed at concentrations of 10 nmol/mg from ethyl linoleate, 32 nmol/mg from ethyl linolenate, and 50 nmol/mg from ethyl arachidonate. MG was also formed from the ethyl esters of fatty acids. MA and acrolein were formed from squalene, cod liver oil, and ethyl esters of fatty acids under the same conditions. Glyoxal and MG were produced from acetaldehyde, acrolein, and propanal in the range 2–9 nmol/mg. Prior to this study, glyoxal and MG had never been reported as photodegradation products of lipids or fatty acids.

Excessive exposure of skin to ultraviolet (UV) radiation is linked with biological damage such as sunburn, inflammation, and perhaps aging and skin cancer (Gange and Rosen, 1986). Lipid peroxidation caused by UV irradiation may play a role in these toxic effects because of the production of reactive carbonyls including formaldehyde, acrolein, malonaldehyde (MA), glyoxal, and methylglyoxal (MG).

Among the carbonyl compounds produced, glyoxal and MG reportedly have potent mutagenic and cytogenic activities (Kasai and Nishimura, 1986; Ueno et al., 1991). It is reported that 50% of coffee's mutagenicity is due to the presence of MG (Kasai et al., 1982). Glyoxal is present in numerous materials such as ozonated drinking water (Glaze et al., 1989), cigarette smoke (Moree-Testa and Saint-Jalm, 1981), and heated glucose (Kasai and Mishimura, 1986). MG has been found in bread, boiled potatoes (Kajita and Senda, 1972), coffee, soy sauce, root beer, wine, apple juice, tomato juice, and tea (Hayashi and Shibamoto, 1985).

Analysis of glyoxal and MG is difficult because they are water soluble and polymerize readily. The most commonly used method to analyze glyoxal or MG includes derivatization. For example, glyoxal and MG were analyzed as a quinoxaline derivative in cigarette smoke (Moree-Testa and Saint-Jalm, 1981) and in rat tissues (Ohmori et al., 1987). There are also reports determining glyoxal and MG formed from carbohydrates in foods. There are, however, no reports on their formation from lipids.

In the present study, glyoxal and MG formed from lipids and volatile aldehydes upon UV irradiation were analyzed as quinoxaline and 2-methylquinoxaline (2-MQ), respectively.

MATERIALS AND METHODS

Materials. Glyoxal (40% in water), MG (40% in water), o-phenylenediamine (o-PDA)/HCl, propanal, 2-methylpyrazine, acetaldehyde, 2-isobutylthiazole, 2,4,5-trimethylthiazole, and cysteamine/HCl were purchased from Aldrich Chemical Co. (Milwaukee, WI). Ethyl linoleate, ethyl linolenate, ethyl arachidonate, cod liver oil, acrolein, squalene, tetrahydrofuran (THF), pyridine, 6-methyl-5-hepten-2-one, and acetaldehyde were obtained from Sigma Chemical Co. (St. Louis, MO). *N*-Methylhydrazine was bought from Fluka Chemical Corp. (Ronkonkoma, NY). A stock solution of GC internal standard for acrolein, malonaldehyde (MA), glyoxal, and MG was prepared by adding 15 mg of 2-methylpyrazine to 100 mL of dichloromethane (150 μg/mL). The internal standard for formaldehyde and acetaldehyde was prepared by adding 25 mg of 2,4,5-trimethylthiazole to 25 mL of dichloromethane (1 mg/mL) and 25 mg of 2-isobutylthiazole to 25 mL of dichloromethane (1 mg/mL). Standard 1-methylpyrazoline and 1-methylpyrazole were prepared as described previously (Yasuhara et at., 1989).

Synthesis of Quinoxaline Standard. Quinoxaline was synthesized according to the method reported previously (Moree-Testa and Saint-Jalm, 1981) with minor modification. Glyoxal in 20 mL of 60% ethanol solution (0.44 mmol/mL) was added gradually into 20 mL of o-PDA/HCl (9.67 mmol) aqueous solution over a period of 45 min. The pH of the o-PDA solution was adjusted to 7.5–8.0 with 1 N NaOH prior to the reaction. The resulting solution was stirred with a magnetic stirrer for an additional 1 h at room temperature. The reaction mixture was extracted with 50 mL of dichloromethane three times. The extract was dried over anhydrous sodium sulfate for 30 min and then filtered. After removal of dichloromethane in vacuo, crude quinoxaline was recovered with 95% yield. It was further purified by a silica gel column (20 cm × 1.5 cm i.d.) with 30 mL each of hexane and ethyl acetate in series; 99.9% purity was obtained. The structure of the product was confirmed as quinoxaline by mass spectra, ^{1}H NMR, and ^{13}C NMR: ^{1}H NMR ($CDCl_3$, δ 7.26) δ 7.50 (2 H, dd, J = 7.5, 3.0 Hz, aromatic H's), 7.90 (2 H, dd, J = 7.5, 3.0 Hz, aromatic H's), 8.61 (1 H, s, N=CH); ^{13}C NMR ($CDCl_3$, δ 77.71) δ 130.03, 130.37, 143.07, 145.12. GC/MS spectra have been previously reported (Moree-Testa and Saint-Jalm, 1981).

Synthesis of 2-MQ Standard. 2-MQ was synthesized according to the method reported previously (Moree-Testa and Saint-Jalm, 1981) with minor modification. Thirty milliliters of 60% ethanol solution of MG (0.22 mmol/mL) was added gradually into 30 mL of aqueous solution containing 1.35 g of o-phenylenediamine (o-PDA)/HCl and 2.2 g of anhydrous sodium acetate (pH 3.5) over a period

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of 45 min. The rest of the procedure was the same as that for glyoxal. The reaction was also performed at pH 6 and 9. Crude 2-MQ was further purified by a silica gel column with hexane and ethyl acetate. Over 98% pure 2-MQ was obtained. The structure of the product was confirmed as 2-MQ by mass spectra, ^{1}H NMR, and ^{13}C NMR: ^{1}H ($CDCl_3$, δ 7.26) δ 2.38 (s, 3 H, CH_3), 7.34 (m, 2 H, aromatic H's), 7.71 (m, 2 H, aromatic H's), 8.34 (s, 1 H, N=CH); ^{13}C ($CDCl_3$, δ 77.62) δ 22.7, 128.81, 129.01, 129.30, 130.08, 140.03, 142.12, 146.12, 153.86. Mass spectra of 2-MQ have been previously reported (Moree-Testa and Saint-Jalm, 1981).

Photoirradiation of Squalene, Fatty Acid Esters, and Volatile Aldehydes. Squalene (43 mg) was dissolved in 2 mL of dichloromethane and coated on the inside of 20 cm × 2 cm i.d. Pyrex tubes. Coating was achieved by evaporating the solvent under a nitrogen stream while the tubes were rotated. Ethyl linoleate (8.5 mg), ethyl linolenate (8.9 mg), ethyl arachidonate (8.4 mg), cod liver oil (9.0 mg), acetaldehyde (7.9 mg), acrolein (8.4 mg), propanal (8.1 mg), and 6-methyl-2-hepten-2-one (8.5 mg) were also coated on the inside wall of 15 cm × 1.5 cm i.d. Pyrex tubes. Sample tubes were sealed and then irradiated in a Rayonet RRR-100 chamber reactor equipped with eight UVB (λ = 300 nm) lamps for various times. Lamp intensity was measured near the center of the reactor with Spectroline UVB meters (Spectronics Corp., Westbury, NY) at 2.2 ± 0.4 mW/cm^2 (mean ± SD, n = 4). Control sample tubes were wrapped with an aluminum foil and kept at −5 °C.

Analysis of Photoproducts. o-PDA (150 μL) in THF (5 mL) was added to the samples to derivatize glyoxal and MG to quinoxaline and 2-MQ, respectively. Each solution was adjusted to pH 6.5–7.5 with 0.1 N NaOH and then stirred with a magnetic stirrer for 1.5 h at room temperature. The volume of each solution was brought to 10 mL with absolute ethanol, and 150 μL of 2-methylpyrazine stock solution was added as a GC internal standard. A sample was analyzed for quinoxaline and 2-MQ by GC with a nitrogen–phosphorus detector (NPD). GC standard curves for quinoxaline and 2-MQ were prepared by the method reported previously (Ettre, 1967).

MA and acrolein were derivatized to 1-methylpyrazole and 1-methyl-2-pyrazoline, respectively, and then analyzed by a GC equipped with an NPD according to the method reported previously (Umano et al., 1988).

Formaldehyde, acetaldehyde, and propanal were derivatized to corresponding thiazolidines and then analyzed by a GC with an FPD according to the method reported previously (Hayashi et al., 1985).

Instrumental Analysis. A Hewlett-Packard (HP) Model 5890 GC equipped with an NPD and a 30 m × 0.25 mm i.d. bonded-phase DB-Wax fused silica capillary column (J&W Scientific, Folsom, CA) was used to analyze quinoxaline, 2-MQ, 1-methylpyrazole, and 1-methyl-2-pyrazoline. The GC peak areas were integrated with an HP 3396 Series II integrator. The oven temperature was held at 80 °C for 3 min and then programmed to 180 °C at 4 °C/min. Injector and detector temperatures were 250 and 300 °C, respectively. The linear velocity of helium carrier gas was 28 cm/s with a split ratio of 25:1.

An HP 5890 GC equipped with an FPD and a 30 m × 0.25 mm i.d. bonded-phase DB-1 fused silica capillary column (J&W Scientific) was used to analyze thiazolidine, 2-methylthiazolidine, and 2-ethylthiazolidine. The GC peak areas were integrated with a Spectra-Physics SP 4290 integrator. The oven temperature was programmed from 50 to 200 °C at 8 °C/min. Injector and detector temperatures were 220 and 230 °C, respectively. The linear velocity of helium carrier gas was 33 cm/s with a split ratio of 34:1.

Table I. Amounts of Glyoxal and MG Formed in Squalane by UV Irradiation for Different Time Periods

time, h	amount,[a] nmol/mg of squalene	
	glyoxal	MG
2	1.22 ± 0.16	2.42 ± 0.37
4	2.92 ± 0.21	5.57 ± 0.37
6	4.84 ± 0.55	8.46 ± 063
8	9.24 ± 0.23	14.00 ● 0.46
10	9.59 ± 0.21	14.41 ± 0.48
12	6.70 ± 0.46	10.42 ± 0.71
15	3.87 ± 0.74	6.88 ± 0.91

[a] Values are mean ± standard deviation (n = 5) and corrected for controls.

An HP 5890 GC interfaced to a VG Trio II mass spectrometer with a VG II-250 computer data system was used for mass spectrometric confirmation of the derivatives. The ionization voltage was 70 eV, and the ion source temperature was 150 °C. The column and oven conditions for GC/MS were as described for the GC analyses.

^{1}H and ^{13}C NMR spectra were recorded with a General Electric QE-300 instrument at 300 and 75.5 MHz, respectively. ^{1}H and ^{13}C chemical shifts are reported in parts per million upfield from residual $CHCl_3$ at δ 7.26 in $CDCl_3$ which was used as solvent. For ^{13}C NMR spectra $CDCl_3$ was used as an internal standard and solvent with chemical shift of δ 76.9.

RESULTS AND DISCUSSION

The irradiation system in the present study was chosen as a simple model for photochemical reactions that might occur in lipids upon sunlight exposure. UVB lamps used were not exactly same spectrum as sunlight. However, their output closely resembles the solar UV spectrum at the earth's surface (Rabek, 1982).

For analysis of glyoxal and MG, the present method did not require strong acidic conditions in contrast to the previous procedure (Ohmori et al., 1987). Mild conditions for derivatization are essential because any severe conditions, such as a strong acidic solution, may alter the chemical of interest.

The yields of 2-MQ from the reaction of MG with o-PDA at pH 3.5, 6, and 9 were 53.0%, 96.0%, and 99.8%, respectively. THF gave the best result as a reaction solvent among the solvents used including diethyl ether, dichloromethane, ethanol, and propanol.

Table I shows the amounts of glyoxal and MG produced from squalene irradiated by UV light for various periods of time. Figure 1 shows a typical gas chromatogram of a sample from squalene irradiated by UV light. Trace amounts of glyoxal (0.16 nmol/mg) and MG (0.32 nmol/mg) were already present in control samples. Maximum amounts of glyoxal (9.59 nmol/mg) and MG (14.41 nmol/mg) were formed after 10 h of UV irradiation. Their concentration declined after 10 h of UV irradiation. Glyoxal and MG have not been reported as photooxidation products of squalene prior to the present study. Squalene concentrations are higher in human skin surface lipid (10%) than in other body tissues (0.5% maximum) (Singer and Pittz, 1985). The present studies demonstrate that toxic aldehydes are formed from squalene upon UV irradiation. The reactivity of these products may be associated with their carcinogenic activity as a result of adduct formation with deoxyribonucleic acid (DNA) (Goldschmidt, 1984). Basu et al. (1988) reported that a

Table II. Amounts of Low Molecular Weight Carbonyl Compounds Formed from Various Lipids Irradiated by UV Light for 6 h

lipid	amount,[a] nmol/mg of lipid			
	MA	acrolein	glyoxal	MG
ethyl linoleate	8.56 ± 1.00	0.51 ± 0.08	10.43 ± 1.73	0.92 ± 0.18
ethyl linolenate	44.51 ± 1.30	1.51 ± 0.20	31.66 ± 2.38	3.62 ± 0.17
ethyl arachidonate	59.40 ± 0.35	1.65 ± 0.21	49.53 ± 0.65	7.28 ± 0.23
cod liver oil	69.50 ± 14.9	10.90 ± 3.06	27.23 ± 2.64	5.72 ± 0.33

[a] Values are mean ± standard deviation (n = 5) and corrected for controls.

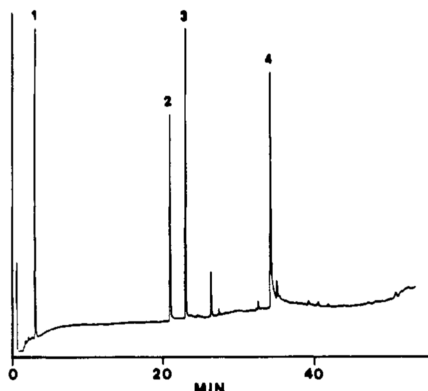

Figure 1. Typical gas chromatogram of a reaction mixture of irradiated squalene and o-PDA. 1, Internal standard (2-methylpyrazine); 2, quinoxaline (from glyoxal); 3, 2-MQ (from MG); 4, unreacted o-PDA.

series of acrolein derivatives including MG readily formed adducts with guanine and guanine nucleosides. Glyoxal and MG were shown to be potent mutagenic and cytogenic substances (Furihata et al., 1989; Ueno et al. 1991). For example, DNA damage in pyloric mucosa of the stomach of male F344 rats was demonstrated following oral administration of glyoxal (Furihata et al., 1989). In light of the above information, MG formation from squalene at the skin surface may have implications of some toxicological consequence. MA (5.49 nmol/mg) and acrolein (0.86 nmol/mg) were also found in squalene after UV irradiation for 6 h.

Table II shows the levels of MA, acrolein, glyoxal, and MG found in ethyl linoleate, ethyl linolenate, ethyl arachidonate, and cod liver oil irradiated by UV for 6 h. The greatest amounts of MA were formed in cod liver oil (69.50 nmol/mg) followed by ethyl arachidonate, ethyl linolenate, and ethyl linoleate. As proposed previously, higher levels of MA production were attributed to the increasing number of double bonds in the substrate (Pryor et al., 1976). Approximately 70% of cod liver oil consists of ω-3 polyunsaturated fatty acids, including octadecatetraenoic acid, eicosapentenoic acid, and docosahexenoic acid. However, glyoxal and MG were formed in the greatest quantity from ethyl arachidonate, suggesting that the formation mechanisms of glyoxal and MG are different from those of MA or acrolein.

Acrolein levels were significantly higher in cod liver oil than in other samples. Acrolein is reportedly formed from glycerol, which can be produced from hydrolysis of a fatty acid glyceride (Umano et al., 1987). Only cod liver oil contained glycerol among the chemicals used. Formation of acrolein from ethyl linolenate and ethyl arachidonate upon UV irradiation was previously reported (Dennis and Shibamoto, 1990) but not from squalene and ethyl linoleate. The amounts of acrolein found were, however, considerably less than those of MA, glyoxal, and MG in all samples. This may be due to its secondary reaction to form other compounds such as formaldehyde, glyoxal, and MG. When acetaldehyde, acrolein, and propanal were

Table III. Amounts of Glyoxal and MG Formed from Low Molecular Weight Carbonyl Compounds Irradiated by UV Light for 4 h

chemical	amount,[a] nmol/mg of chemical	
	glyoxal	MG
acetaldehyde	8.22 ± 1.63	2.17 ± 0.26
acrolein	7.73 ± 0.94	2.38 ± 0.34
propanal	3.33 ± 0.48	7.97 ± 0.85
acetone	2.43 ± 0.19	9.20 ± 1.41

[a] Values are mean ± standard deviation (n = 5) and corrected for controls.

irradiated by UV light for 4 h, significantly high levels of glyoxal and MG formed (Table III). In addition to glyoxal and MG, formaldehyde, acetaldehyde, and propanal were produced from acrolein in the levels of 106.54 ± 10.62 (n = 5), 62.23 ± 6.99 (n = 5), and 28.01 ± 0.42 nmol/mg (n = 5), respectively. These results indicate that acrolein underwent further reactions to form secondary products.

Many proposed mechanisms for lipid peroxidation have been reported. For example, MA was proposed to form from the decomposition of prostaglandin-like endoperoxide intermediates (Pryor et al., 1976). Acetone and formaldehyde were proposed to form through 6-methyl-5-hepten-2-one from squalene upon UV irradiation (Yeo and Shibamoto, 1992). In fact, when 6-methyl-5-hepten-2-one was irradiated by UV light for 8 h, 3.17 ± 0.24 nmol/mg (n = 5) of glyoxal and 6.69 ± 0.21 nmol/mg (n = 5) of MG were formed in the present study. Most proposed mechanisms require, however, many steps to form low molecular weight carbonyl compounds including formaldehyde, acetaldehyde, propanal, acetone, MA, acrolein, glyoxal, and MG. The results of the present study suggest that many low molecualr weight radicals such as •OH, •CHO, $\cdot CH_2CHO$, $\cdot CH_3$, and $\cdot COCH_3$ are formed in the early stage of photoirradiation and that these radicals combine to form low molecular weight carbonyl compounds.

LITERATURE CITED

Basu, A. K.; O'Hara, M. S.; Valladier, P.; Stone, K.; Moles, O.; Marnett, L. Identification of Adducts Formed by Reaction of Guanine Nucleosides with Malondialdehyde and Structurally Related Aldehydes. *Chem. Res. Toxicol.* **1988**, *1*, 53–59.

Dennis, K. J.; Shibamoto, T. Gas Chromatographic Analysis of Reactive Carbonyl Compounds Formed from Lipids upon UV-Irradiation. *Lipids* **1990**, *25*, 460–464.

Ettre, L. S. Interpretation of Analytical Results. In *The Practice of Gas Chromatography*; Ettre, L. S., Zlatkis, A., Eds.; Interscience Publishers: New York, 1967; p 402.

Furihata, C.; Hatta, A.; Sato, Y.; Matsushima, T. Alkaline Elution of DNA from Stomach Poloric Mucosa of Rats Treated with Glyoxal. *Mutat. Res.* **1989**, *213*, 227–231.

Gange, R. W.; Rosen, C. F. UVA Effects on Mammalian Skin and Cells. *Photochem. Photobiol.* **1986**, *46*, 701–704.

Glaze, W. H.; Koga, M.; Cancilla, D. Ozonation Byproducts. 2. Improvement of an Aqueous-Phase Derivatization Method for the Detection of Formaldehyde and Other Carbonyl Com-

pounds formed by the Ozonation of Drinking Water. *Environ. Sci. Technol.* **1989**, *23*, 838–847.

Goldschmidt, B. A. Role of Aldehydes in Carcinogenesis. *J. Environ. Sci. Health, Part C: Environ. Carcinog. Rev.* **1984**, *C2*, 231–249.

Hayashi, T.; Shibamoto, T. Analysis of Mutagenic Methyl Glyoxal in Foods and Beverages. *J. Agric. Food Chem.* **1985**, *33*, 1090–1093.

Hayashi, T.; Reece, C. A.; Shibamoto, T. A New Analytical Method for Volatile Aldehydes. In *Characterization and Measurement of Flavor Compounds*; Bills, D. D., Mussinan, C. J., Eds.; ACS Symposium Series 289; American Chemical Society: Washington, DC, 1985; pp 61–78.

Kajita, T.; Senda, M. Simultaneous Determination of L-Ascorbic Acid, Triose Reductose, and Their Related Compounds in Foods by Polarographic Method. *Nippon Nogei Kagaku Kaishi* **1972**, *46*, 137–145.

Kasai, H.; Nishimura, S. Hydroxylation of Guanine in Nucleosides and DNA at the C-8 Position by Heated Glucose and Oxygen Radical Forming Agents. *Environ. Health Perspect.* **1986**, *67*, 111–116.

Kasai, H.; Kumeno, K.; Yamaizumi, Z.; Nishimura, S.; Nagao, M.; Fujita, Y.; Sugimura, T.; Nukaya, H.; Kosuge, T. Mutagenicity of Methylglyoxal in Coffee. *Gann* **1982**, *73*, 681–684.

Moree-Testa, P.; Saint-Jalm, Y. Determination of α-Dicarbonyl Compounds in Cigarette Smoke. *J. Chromatogr.* **1981**, *217*, 197–208.

Ohmori, S.; Kawase, M.; Mori, M.; Hirota, T. Simple and Sensitive Determination of Methylglyoxal in Biological Samples by Gas Chromatography with Electron Capture Detection. *J. Chromatogr.* **1987**, *415*, 221–229.

Pryor, W. A.; Stanley, J. P.; Blair, E. Autoxidation of Polyunsaturated Fatty Acids: II. A Suggested Mechanism for the Formation of TBA-Reactive Materials from Prostaglandin-Like Endoperoxides. *Lipids* **1976**, *11*, 370–379.

Rabek, J. F. Optical Radiation Sources. In *Experimental Methods in Photochemistry and Photophysics, Part I*; Rabek, J. F., Ed.; Wiley: Chichester, U.K., 1982; Chapter 3.

Singer, E. J.; Pittz, E. P. Interaction of Surfactants with Epidermal Tissues: Biochemical and Toxicological Aspects. In *Surfactants in Cosmetics*; Rieger, M. J., Ed.; Dekker: New York, 1985; pp 133–194.

Ueno, H.; Nakamuro, K.; Sayato, Y.; Okada, S. DNA Lesion in Rat Hepatocytes Induced by in vitro and in vivo Exposure to Glyoxal. *Mutat. Res.* **1991**, *260*, 115–119.

Umano, K.; Shibamoto, T. Analysis of Acrolein from Heated Cooking Oils and Beef Fat. *J. Agric. Food Chem.* **1987**, *35*, 909–912.

Umano, K.; Dennis, K. J.; Shibamoto, T. Analysis of Free Malondialdehyde in Photoirradiated Corn Oil and Beef Fat via a Pyrazole Derivative. *Lipids* **1988**, *23*, 811–814.

Yasuhara, A.; Dennis, K. J.; Shibamoto, T. Development and Validation of New Analytical Method for Acrolein in Air. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 749–751.

Yeo, H. C. H.; Shibamoto, T. Formation of Formaldehyde and Malonaldehyde by Photooxidation of Squalene. *Lipids* **1992**, *27*, 50–53.

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