Synthesis, Characterization, and Potential Use of 2-Dodecylcyclobutanone as a Marker for Irradiated Chicken

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The synthesis and characterization of 2-dodecylcyclobutanone is described. Solvent extraction techniques for the isolation of this compound from irradiated minced chicken meat and its detection by selected ion monitoring are outlined. The compound was not detected in either raw or cooked nonirradiated minced chicken meat by the methods used, but its presence was confirmed in the irradiated samples. 2-Dodecylcyclobutanone was detectable for 20 days postirradiation. The dose (4.7 kGy) of irradiation applied was below the recommended upper limit for food (10 kGy), and this compound may have potential as a marker for irradiated chicken meat and for other foods containing lipid.

INTRODUCTION

The use of ionizing radiation for the preservation of food has been under investigation for many years but has yet to receive worldwide acceptance. Although the process of irradiation can be controlled by good management at the irradiation facility and the routine use of dosimeters, it is generally accepted that the development of a test or tests for the detection of irradiated food would facilitate international trade and enhance consumer confidence in the existing control procedures.

To data, a number of methods that may prove useful for the detection of irradiated food have been investigated. These include electron spin resonance spectroscopy to detect free radicals in bone (Lea et al., 1988; Stevenson and Gray, 1989a,b), thermoluminescence of spices (Heide and Bogl, 1988; Sanderson et al., 1989), formation of o-tyrosine in meat (Meier et al., 1989), and the production of volatile compounds from irradiated fats (Nawar, 1988). The major classes of volatile compounds produced by irradiation of fats are hydrocarbons, aldehydes, methyl and ethyl esters, and free fatty acids. According to Nawar and Balboni (1970), the composition of the products formed can be predicted to some extent if the fatty acid composition of the fat is known. As well as these major classes of compounds, LeTellier and Nawar (1972), using simple triglycerides irradiated under vacuum at 60 kGy, also isolated a series of cyclic compounds which originally appeared to be unsaturated aldehydes but were later shown to be cyclobutanones containing the same number of carbons as the parent fatty acid. They were substituted with an alkyl group located in the 2-position. More recently, Handel and Nawar (1981) also isolated 2-dodecylcyclobutanone from a synthetic phospholipid irradiated at 500 kGy. The procedure for isolation of all these volatile compounds involved vacuum distillation and analysis by gas chromatography (Nawar et al., 1969).

The objective of the present experiment was to synthesize and characterize 2-dodecylcyclobutanone, to devise a solvent extraction system for the isolation of this cy-

clobutanone from irradiated chicken meat and skin, and to confirm its presence in these samples by using gas chromatography–mass spectrometry. In the first instance, it was decided to concentrate solely on 2-dodecylcyclobutanone as this is formed from triglycerides containing palmitic acid, a fatty acid found in substantial quantities in chicken meat. Preliminary results have been reported by Stevenson et al. (1990).

EXPERIMENTAL METHODS

Gas Chromatography–Mass Spectrometry (GC–MS). Separations were performed on a Hewlett-Packard 5890 GC directly linked to a Hewlett-Packard 5970 mass selective detector (MSD) and equipped with a Hewlett-Packard 7673A auto sampler. This apparatus was controlled by a Hewlett-Packard 300 series computer. Two capillary columns were used: an Ultra 1 (Hewlett-Packard, Winnersch, U.K.) 12 m × 0.22 mm (i.d.) with a 0.33-μm stationary phase (100% dimethyl polysiloxane) in experiment I and an Ultra II (as for Ultra 1 except stationary phase is 5% diphenyl, 95% dimethyl, polysiloxane) in experiments II and III. Conditions used were as follows: injector temperature, 250 °C; transfer line temperature, 225 °C; initial column oven temperature, 55 °C for 1 min; first ramp, 15 °C/min to 200 °C; second ramp, 25 °C/min to 300 °C, final temperature held for 5 min; injection volume, 2 μL; injection mode split 20:1; solvent delay, 4 min; multiplier voltage, 2000 V; carrier, He, 1 mL/min. The MSD was operated in two modes: full scan 30–500 amu and selected ion monitoring of ions m/z 98 and 112 using a dwell time of 50 ms/mass.

The chemical impact (CI) mass spectrum of 2-dodecylcyclobutanone was recorded by using a Kratos MS25RFA mass spectrometer (Kratos, Manchester, U.K.), with methane as the reagent gas.

Nuclear Magnetic Resonance Spectroscopy (NMR). The ^{1}H NMR and ^{13}C NMR spectra were recorded on GE-QE 300 and GN-Omega 500-MHz spectrometers respectively, using deuteriochloroform as solvent. Chemical shifts are quoted relative to tetramethylsilane as internal standard; coupling constants are given in hertz.

Infrared Spectroscopy (IR). Spectra were recorded on a Perkin-Elmer 983G instrument using potassium bromide disks for solids.

Irradiation Facility. Irradiation was carried out in a Gamma Beam 650 (Nordion International Inc., Kanata, Canada) using cobalt-60 as the source of ionizing radiation.

Dosimetry. Amber perspex dosimeters (United Kingdom Atomic Energy Authority, Type 3402B) were attached to the outside of the samples. After irradiation, the absorbance of the dosimeters at 603 nm was measured in a Pye Unicam SP8-100

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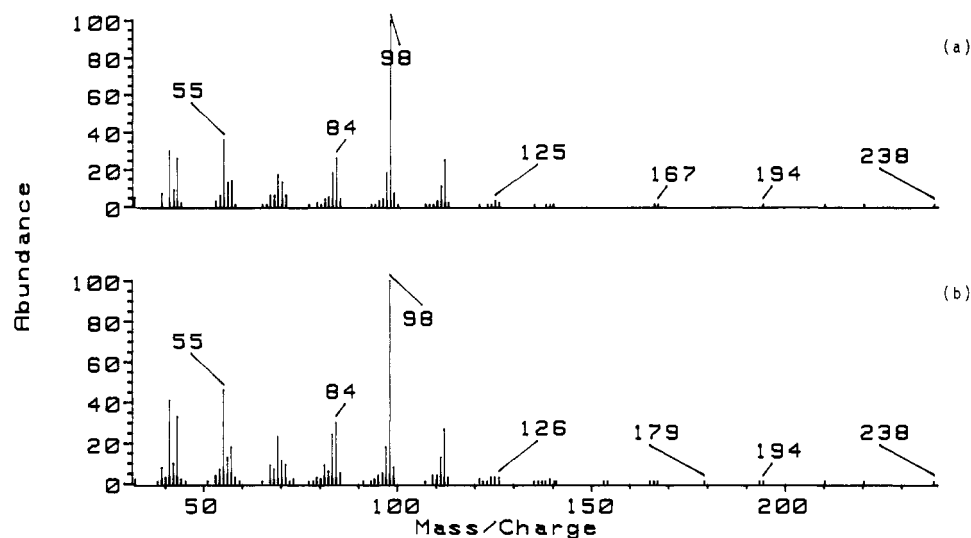
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Figure 1. Electron impact mass spectrum of standard 2-dodecylcyclobutanone (a) and the corresponding spectrum for this compound extracted from irradiated (4.3 kGy) chicken skin (b).

UV–vis spectrometer and their thickness measured in a digital electronic micrometer (RS Components Ltd., Corby, U.K.). The corresponding dose received was then obtained from a calibration graph provided by the National Physical Laboratory, Teddington, U.K.

Reagents. All reagents were used as purchased from standard commercial sources with the following exceptions: Diethyl ether stored over sodium wire was distilled prior to use. Technical grade calcium stearate (BDH Chemicals Ltd.) was stirred for 2 h in acetonitrile and filtered and this washing repeated with hexane and then diethyl ether. The residue was dried in an oven at 100 °C overnight. Florisil mesh 60–100 PR (Sigma Chemical Co., St. Louis) was deactivated by the addition of distilled water, in proportions 20 mL to 100 g of Florisil and the mixture left to equilibrate overnight. The internal standard used was 2-cyclohexylcyclohexanone (Fluka Chemicals Ltd., Glossop, U.K.), which eluted before 2-dodecylcyclobutanone and produced a m/z 98 ion.

Synthesis of 2-Dodecylcyclobutanone. The method was similar to the procedure outlined by Miller and Gadwood (1989) for the synthesis of 2-substituted cyclobutanones with the following modifications. The key reagent 1-bromo-1-ethoxycyclopropane (3.3 g, 20 mmol) was prepared by a two-step reaction from ethyl 3-chloropropionate (Miller and Gadwood, 1989; Salaun and Marguerite, 1984) and reacted with tridecanal (2.47 g, 12.5 mmol) to yield the intermediate 1-ethoxy-1-(1′-hydroxytridecyl)-cyclopropane (3.15 g, 55 % yield). This was a high-boiling viscous oil which solidified on refrigeration to give a low melting point solid (mp 39–41 °C). Traces of impurity in this oil could not be readily removed, and thus the product was characterized by IR, MS, and NMR methods and used without purification in the next stage.

1-Ethoxy-1-(1′-hydroxytridecyl)cyclopropane. MS Anal. Calcd for $C_{18}H_{36}O_2$: 284.2715. Found: 284.2728. MS m/z 285 (M + 1, 8%), 284 (M⁺, 14), 57 (M − 227, 100); IR (neat) 3500 (w), 2960 (s), 1700 (w), 1460 (m) cm⁻¹; ¹H NMR (500 MHz, CDCl₃) 0.60 (2 H, m, 2-H or 3-H), 0.81 (2 H, m, 2-H or 3-H), 0.89 (3 H, t, J = 7.5 Hz, Me), 1.14 (3 H, t, J = 7.0 Hz, OCH₂Me), 1.26 (18 H, m, CH₂ × 9), 1.50 (2 H, m, CH₂CHOH), 3.48 (1 H, m, CHOH), 3.50 (1 H, dq, J = 9.5 and 7.0 Hz, 2′-H), 3.72 (1 H, dq, J = 9.5 and 7.0 Hz, 2′-H).

1-Ethoxy-1-(1′-hydroxytridecyl)cyclopropane (3.0 g, 10.6 mmol) was allowed to stir in ether and fluoboric acid (5.0 mL, 48% aqueous) for 4 days, allowing rearrangement to take place. After vacuum distillation, the product (0.93 g, 37% yield) was further purified by flash chromatography using Merck Kieselgel 60 (230–400 mesh) to yield a viscous oil, boiling point 88–96 °C at 0.075 mmHg, which solidified on storage at 5 °C (mp 25–27 °C).

2-Dodecylcyclobutanone. (Found: C, 80.2; H, 12.5. $C_{16}H_{30}O$ requires C, 80.7; H, 12.6%). EI-MS (Figure 1a) CI-MS, m/z 239 (M + 1, 39%); IR 2960 (s), 2850 (s), 1780 (s), 1460 (m), 1090 (w) cm⁻¹; ¹H NMR (500 MHz, CDCl₃) 0.88 (3 H, t, J = 7.5 Hz,

Me), 1.26–1.36 (20 H, m, CH₂ × 10), 1.49 (1 H, m, 1′-H), 1.66 (2 H, m, 1′-H, 3-H), 2.17 (1 H, m, 3-H), 2.90 (1 H, m, 4-H), 3.05 (1 H, m, 4-H), 3.28 (1 H, m, 2-H); δ_C (125 MHz, CDCl₃) 14.12 (CH₃), 16.88 (3-C), 22.67 (11′-C), 27.00 (1′-C), 29.35–29.73 (2′-C, 3′-C, 4′-C, 5′-C, 6′-C, 7′-C, 8′-C, 9′-C), 31.91 (10′-C), 44.38 (4-C), 60.62 (2-C), 212.10 (1-C).

Extraction Procedure I. Samples (10 g) of minced chicken meat were extracted according to the method of Schultz et al. (1971) but with the following modification. After filtration of the added calcium stearate, a 20-mL portion of the filtrate was poured into a stoppered tube and 5 mL of hexane added. The contents of the tube were mixed, and the hexane layer was pipetted off and retained. This hexane extraction step was repeated twice more, and the combined hexane layers were evaporated to dryness under nitrogen. The residue was resuspended in 1 mL of hexane containing the internal standard, at a concentration of 0.5 μg/mL and analyzed by GC–MS.

Extraction Procedure II. Lipid was extracted from 10-g samples of minced chicken meat or skin as described in procedure I to the stage where ether was evaporated to dryness. The residue was resuspended in a small volume of hexane (1–2 mL) and lipid removed by using a column (20 mm × 300 mm) of deactivated Florisil. The extract was eluted with 1% diethyl ether in hexane, evaporated to dryness, and resuspended in 200 μL of hexane for application to GC–MS.

Experiment I. One whole frozen chicken carcass was bandsawed, minced, and mixed to provide a homogeneous bulk sample. Approximately 40-g portions were weighed into eight screwtop glass vials (48 mL) fitted with Teflon seals. The remaining minced chicken was stored at −20 °C and subsampled as required to provide nonirradiated samples and samples spiked with the reference standard, 2-dodecylcyclobutanone (4 μg in 200 μL of hexane) prior to extraction. The vials of minced chicken meat were given an average dose of 4.7 (±0.1) kGy (dose rate 21 kGy/h; irradiation temperature 12 °C) and stored at 5 °C. Triplicate samples were extracted according to procedure I on the day of irradiation and 2, 5, 7, 9, 12, 14, and 20 days postirradiation. An unirradiated control and spiked sample were extracted on the day of irradiation, and on the following sampling days only a spiked sample was used. The extracts were applied to GC–MS performed in the selected ion monitoring mode.

Experiment II. Following irradiation of one whole fresh chicken carcass at a dose of 4.3 kGy (dose rate 1.3 kGy/h;, irradiation temperature 12 °C), the skin was removed and homogenized in a food processor. Unirradiated chicken skin was also homogenized to provide a control and the lipid from both samples extracted according to procedure II. The extracts were applied to GC–MS performed in both the selected ion monitoring and full scan mode. Splitless injections were used for samples recorded in the full scan mode.

Experiment III. Chicken meat from one carcass was removed from the bone and homogenized to produce a bulk sample. The

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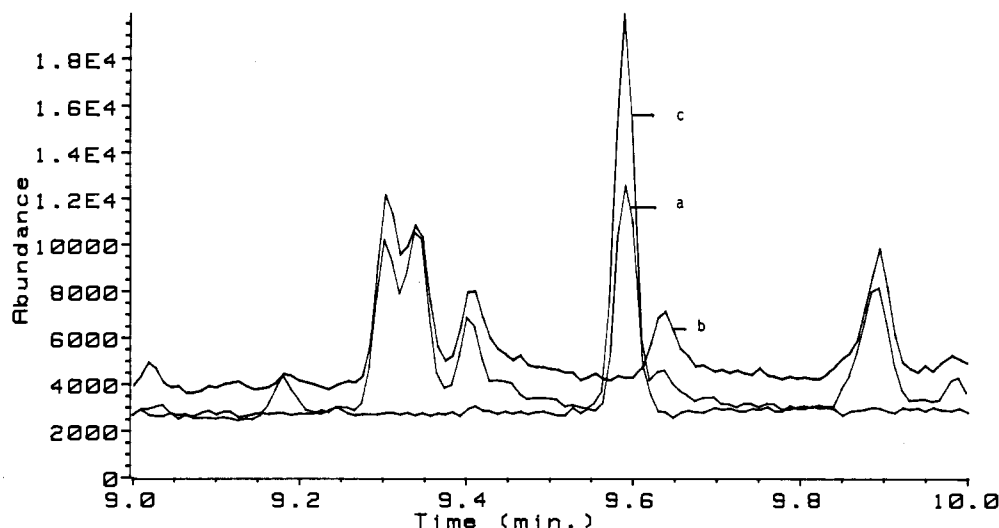*J. Agric. Food Chem.*, Vol. 39, No. 4, 1991 **791**

Figure 2. Selected ion monitoring of the sum of ions 98 and 112 of standard 2-dodecylcyclobutanone (a) and unirradiated (b) and irradiated (4.7 kGy) minced chicken meat (c).

homogenized meat was placed in plastic containers and frozen at −20 °C until required for analysis. Each day, over a 6-day period, one sample was thawed and portioned to provide unirradiated control, spiked control, cooked, and irradiated samples. The samples for cooking were placed in tinfoil and heated to 200 °C for 1 h, while those for irradiation were prepared as in experiment I and given a dose of 5.1 (±0.70 kGy (dose rate 20 kGy/h; irradiation temperature 12 °C). The spiked samples had 5 μg of 2-dodecylcyclobutanone in 200 μL of hexane added to 10 g of homogenized chicken meat prior to extraction. All samples were extracted according to procedure II and the extracts applied to GC–MS in the selected ion monitoring mode.

RESULTS AND DISCUSSION

The electron impact mass spectrum showed a weak mass ion at 238 m/z and a fragmentation pattern similar to that of 2-ethylcyclobutanone (LeTellier and Nawar, 1972). The chemical ionization mass spectrum gave a strong M + 1 at 239 m/z.

^{1}H NMR assignments were made on the basis of high-resolution (500 MHz) and COSY (2-D correlation spectroscopy) spectra.

^{13}C NMR assignments were based on high-resolution (125 MHz) ^{13}C NMR spectroscopy using the DEPT (distortionless enhancement by polarization transfer) method to assign the multiplicity of ^{13}C signals. Further assignments were made by ^{1}H ^{13}C correlation methods.

Selected ion monitoring of the extract (experiment 1) from the irradiated chicken meat for ions m/z 98 and 112 produced a peak with a retention time and ion ratio which corresponded to that of standard 2-dodecylcyclobutanone (Figure 2). The unirradiated control samples showed no detectable peak at this retention time under the analytical conditions used, while the spiked unirradiated samples all produced a peak at the expected retention time. During the study, the area of the peak from the spiked samples did not increase, confirming that no generation of 2-dodecylcyclobutanone had taken place in the unirradiated sample.

Not only was the presence of 2-dodecylcyclobutanone confirmed in the samples immediately following irradiation but it was still present at a detectable level 20 days postirradiation (Table I). Although there was an increase in the amount of 2-dodecylcyclobutanone between days 0 and 2, it was not statistically significant, and thereafter the amount of compound decreased over the 20-day period. The isolation procedure used in experiment 1 allowed detection and quantification of 2-dodecylcyclobutanone

Table I. Concentration of 2-Dodecylcyclobutanone in Irradiated Minced Chicken Meat Stored for up to 20 Days[a]

storage day of sampling	2-dodecylcyclobutanone, μg/g of fresh chicken	storage day of sampling	2-dodecylcyclobutanone, μg/g of fresh chicken
0	0.244	12	0.219
2	0.292	14	0.230
5	0.280	20	0.180
7	0.276	SEM	0.0238
9	0.262	significance	**

[a] Values are the mean of three measurements. ** = $P < 0.01$.

but the sample contained extraneous compounds, the presence of which reduced the sensitivity of the method and limited the lifetime of the GC capillary column. An alternative approach using adsorption chromatography, which was more effective in removing the coextracted lipid material, was then developed and as a result the sensitivity of the method was enhanced. By use of these extraction procedures, 2-dodecylcyclobutanone has been detected in over 90 samples from 23 chickens irradiated at either 2.5, 5.0, or 50 kGy.

The presence of 2-dodecylcyclobutanone was also detected in irradiated chicken skin. The mass spectrum of the peak obtained after splitless injection of the extract (2 μL) of irradiated chicken run in the full scan mode showed that it was an exact match for that of standard 2-dodecylcyclobutanone (Figure 1a,b). This provided further confirmation that the compound produced on irradiation of chicken skin was 2-dodecylcyclobutanone.

Cooking did not generate 2-dodecylcyclobutanone in the unirradiated samples (Figure 3c), but the compound was detected in all the irradiated samples (Figure 3b). LeTellier and Nawar (1972) postulated that a series of cyclobutanones could be formed from irradiated lipids, and it is possible that some of the other peaks which were present in the irradiated samples may be attributed to other cyclobutanones. Cooked samples also gave peaks which were not present in irradiated samples (Figures 3c).

Although 2-alkylcyclobutanones have been isolated from triglycerides (LeTellier and Nawar, 1972) and synthetic phospholipids (Handel and Nawar, 1981), they have not previously been isolated from a complex food matrix such as chicken meat. The procedure adopted in these previous studies (LeTellier and Nawar, 1972; Handel and Nawar, 1981; Nawar et al., 1969) used vacuum distillation following irradiation at doses of 60 kGy and above, which are much
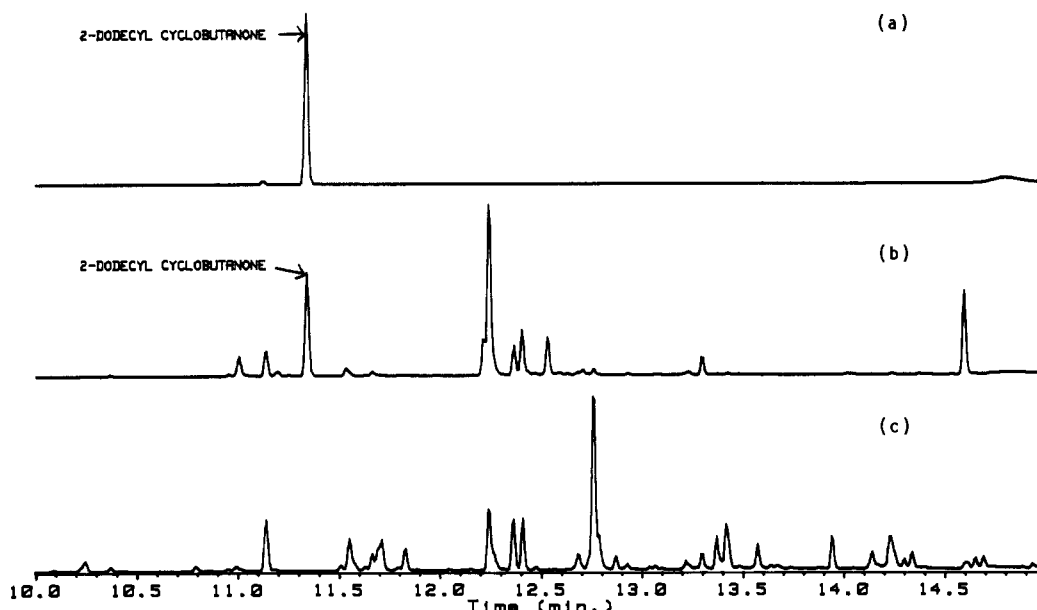
Figure 3. Selected ion monitoring of the sum of ions 98 and 112 of standard 2-dodecylcyclobutanone (a), irradiated (5.1 kGy) (b) and unirradiated cooked minced chicken meat (c).

higher than the recommended upper dose limit for food, namely 10 kGy (ACINF, 1986; JECFI, 1981). These experiments establish that 2-dodecylcyclobutanone can be isolated from minced chicken meat and skin irradiated at a dose below this recommended upper limit of 10 kGy. It is estimated that an irradiation dose of 0.5 kGy could be detected in chicken meat by the present methodology, and this is substantially lower than the 2–2.5-kGy dose of irradiation likely to be used in commercial irradiation of chilled chicken. Also, the extraction procedures described above are simple and with further refinement may form the basis of a routine procedure for isolation of these compounds. The experiments also show that the compounds should be stable for the shelf life of irradiated chilled chicken and are not generated by cooking.

The production of 2-dodecylcyclobutanone shows potential as a postirradiation marker for chicken meat. Further research is required to establish the relationship between the production of this compound and such variables as irradiation dose, dose rate, temperature of irradiation, storage temperature, and length of storage. The possibility of applying the method to a range of foods will also require investigation.

ACKNOWLEDGMENT

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