Identification of oxidation products of solanesol produced during air sampling for tobacco smoke by electrospray mass spectrometry and HPLC†‡

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Solanesol, a 45-carbon, trisesquiterpenoid alcohol found in tobacco leaves and tobacco smoke, has been used as a quantitative marker for tobacco smoke for years. However, solanesol appears to be unreliable as a quantitative marker for tobacco smoke during environmental air sampling because it can be degraded substantially when present as a component of tobacco smoke and by as much as 100% when present as pure solanesol on fortified filters during air sampling. Since there is strong evidence that ozone is the agent responsible for the degradation, solanesol appears to be unreliable as a quantitative marker during indoor air sampling when indoor levels of ozone are greater than about 15 ppb. The degree of loss of pure solanesol is directly proportional to the concentration of ozone and the length of the sampling period and depends on the type of 37 mm membrane filter used for air sampling (PTFE or quartz fiber). While the degree of loss of solanesol is inversely proportional to the relative humidity of the air at a sampling rate of 1.7 L min^{-1}, the degree of loss is virtually independent of relative humidity at a lower sampling rate; *i.e.*, 0.25 L min^{-1}. A curve of loss of solanesol on a filter *versus* concentration of ozone from an ozone generator is virtually identical to a curve segment based on atmospheric ozone under the same conditions of air sampling. Oxidation of solanesol by ozone to approximately 25 to 60% completion produces at least three series of products for a total of at least 26 compounds: (1) isoprenoid acetones, (2) ω-hydroxyisoprenoid acetaldehydes, and (3) isoprenoid oxoaldehydes. All products in each series were tentatively identified as their derivatives with 2-(*p*-aminophenyl)ethanol (APE) by electrospray mass spectrometry (ES-MS). Ten ozonation products were detected as their 2,4-dinitrophenylhydrazine derivatives by HPLC at 360 nm: 4-oxopentanal and nine isoprenoid acetones (acetone, 6-methyl-5-hepten-2-one, geranylacetone, farnesylacetone, tetraprenylacetone, geranylfarnesylacetone, farnesylfarnesylacetone, farnesylgeranylgeranylacetone and bombiprenone.

Introduction

Solanesol is a 45-carbon, trisesquiterpenoid alcohol found in tobacco leaves and tobacco smoke (see Fig. 1). Since airborne solanesol is reported to be very specific for the solid fraction of environmental tobacco smoke (ETS) and since solanesol in solution is measured easily at low levels by HPLC, solanesol has appeared to be an ideal candidate for serving as a quantitative marker for environmental tobacco smoke.[1,2]

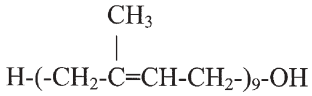

Fig. 1 Molecular structure of solanesol with nine isoprene units (MW = 631.1; CAS RN = 13190-97-1).

The Mine Safety and Health Administration (MSHA) has proposed using the National Institute for Occupational Safety and Health (NIOSH) method 5040 to measure elemental carbon (EC) and organic carbon (OC) for total carbon (TC) on quartz fiber filters as a measure of diesel particulate matter (DPM) in air in mines.[3,4] However, MSHA has been concerned that tobacco smoke from miners may cause a positive bias in the measurement of DPM. Consequently, MSHA has sought the assistance of NIOSH to determine whether solanesol on quartz fiber filters can be measured reliably. MSHA has proposed a change in the exposure standard for DPM from TC to EC due to potential OC interferences.[5]

The American Society for Testing and Materials (ASTM) published in 1998 a sampling and analytical method for solanesol in air for estimating the contribution of tobacco smoke to the total level of respirable suspended particles.[1] Conditions of measuring solanesol in air by the ASTM method are consistent with conditions employed in the present work. Ogden and Maiolo reported average recoveries of 104% and

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† Mention of company names and products does not constitute endorsement by the Centers for Disease Control and Prevention.

‡ Electronic supplementary information (ESI) available: Description of 'ozone generation system for controlled atmospheres' and 'loading membrane filters with tobacco smoke'; supplementary figures (S1–S5) and table (S1). See http://dx.doi.org/10.1039/b505328e

90% for solanesol from filters fortified with known quantities of solanesol by gas chromatography (GC) and HPLC, respectively.[2] However, these authors did not draw environmental air through the fortified filters and did not report the level of solanesol used for fortification.[2] The International Organization for Standardization (ISO) published the same method in 2003.[6]

A literature search has revealed that dozens of articles about air sampling for solanesol in environmental tobacco smoke in many countries have been published in recent years.[7–32] Various groups of researchers have either assumed or concluded that solanesol can be used as a marker for tobacco smoke.[2,18,26,27,29,32–35]

This study evaluates solanesol as a possible quantitative marker for tobacco smoke indoors and outdoors and demonstrates that solanesol does undergo severe losses during air sampling. These observations sparked an investigation into the reason why solanesol was undergoing degradation. Atmospheric ozone, which is ubiquitous,[36] was hypothesized as the agent which was causing degradation of solanesol. In fact, atmospheric ozone is known to cause degradation of terpenoid compounds.[37]

Experimental

Materials

Quartz fiber membrane filters, 37 mm in diameter, were obtained from Pall Life Sciences and Whatman International Ltd. FALP filters (PTFE filters with polyethylene backings), 37 mm in diameter, were obtained from Millipore Corp. Brown, opaque filter cassettes for 37 mm membrane filters, and stainless steel support screens, 100 mesh and 37 mm in diameter, were obtained from SKC Inc. Preweighed filter cassettes containing PVC membrane filters, 37 mm in diameter and 5 μm pore size, cat. No. 711361, were obtained from Mine Safety Appliances Co. S10 LpDNPH cartridges [containing silica gel coated with acidified 2,4-dinitrophenylhydrazine (DNPH)] for carbonyl monitoring were obtained from Supelco.

Solanesol, 90+%, mp = 45.7–47.1 °C, was obtained from Sigma. Also, solanesol, 91.2%, mp = 44.4–45.6 °C, was obtained from Ashian Herbex Ltd., Barkatpura, Hyderabad, India, www.ashianherbex.com. 2-Methylfuran (99%), DNPH (97% and containing 30% water), 6-methyl-5-hepten-2-one (99%), geranylacetone (96%), and hexane (95+%) were obtained from Aldrich Chemical Co. Farnesylacetone, a mixture of *cis* and *trans* isomers (~95%), was obtained from Fluka Chemical Corp. Tetraprenylacetone [3 : 2 (5*E* : 5*Z*) geometrical mixture of (9*E*,13*E*)-6,10,14,18-tetramethyl-5,9,13,17-nonadecatetraen-2-one] was extracted from "Selbex 10%" with hexane and isolated by filtration and evaporation of hexane. "Selbex 10%" is a medical formulation containing 10% tetraprenylacetone by weight and was obtained from Eisai Co., Ltd, Tokyo, Japan. Geranylfarnesylacetone (>98%, 80 : 20 mixture of the all-*E* isomer and the corresponding 5*Z* isomer) was custom synthesized by Chiralix B.V., The Netherlands. 2-(*p*-Aminophenyl)ethanol (APE, 99.5%) was obtained from Chem Service.

4-Oxopentanal bis(DNPH)

4-Oxopentanal bis(DNPH) was synthesized from 2-methylfuran and DNPH in acid solution, washed with deionized water, washed with 95% ethanol, and air dried at room temperature, mp = 230.5–231.8 °C [lit.,[38] mp = 222–225 °C].[38]

Anal. Calcd. for $C_{17}H_{16}N_8O_8$: C, 44.35; H, 3.50; N, 24.34. Found: C, 44.55; H, 3.67; N, 23.81.

DNPH derivative of farnesylfarnesylacetone from tobacco extract

Hexane extract (500 mL) from 25 g of tobacco (from Marlboro cigarettes) in a Soxhlet extraction apparatus was reduced to 10 mL of very dark green-brown solution by boiling off solvent. Each of six S10 LpDNPH samplers was fortified with 600 μL of the concentrated hexane extract. After 4 h of standing, DNPH derivatives from each S10 LpDNPH sampler were eluted with 3 mL of acetonitrile. The orange eluates were combined and concentrated to 4 mL in an oven at 45 °C. HPLC fractions containing farnesylfarnesylacetone-DNPH were collected by using a Deltabond ODS HPLC analytical column, 250 × 3 mm id [part No. 20405-253030, Thermo-Hypersil Keystone], 100% acetonitrile as the mobile phase at a flow rate of 0.7 mL min^{-1} and multiple injections with a volume of 100 μL each. The HPLC fractions were combined and reduced to 4 mL by evaporation of solvent in an oven at 45 °C. The 4 mL of solution was subjected to HPLC by the technique above to collect fractions containing farnesylfarnesylacetone-DNPH a second time. The fractions were combined and reduced to a brown oil by evaporation of solvent at 45 °C in an oven. This oil was submitted to M-Scan (West Chester, PA) for matrix-assisted laser desorption ionization mass spectrometry (MALDI MS). See Fig. S1 in the ESI‡ for the mass spectrum of the sodium adduct.

HPLC

The HPLC was a Waters HPLC system including a Waters 600-MS System Controller and a Waters 486 Tunable Absorbance Detector. For solanesol measurements, the UV detector was set at 205 nm. The analytical column was a Deltabond ODS column, 250 × 3 mm id, packed with 5 μm particles, 12% carbon loading, 300 Å pore size, part No. 20405-253030, from Thermo-Hypersil Keystone. Unless otherwise noted, the mobile phase was 100% acetonitrile, the flow rate was 0.6 mL min^{-1}, and the injection volume was 100 μL. For measurements of all DNPH derivatives, the detector was set at 360 nm. For measurements of DNPH derivatives of carbonyl compounds ranging from formaldehyde to geranylfarnesylacetone (in the isoprenoid acetone series), the analytical column was an ODS Symmetry™ stainless steel cartridge column, 3.9 × 150 mm, containing 5 μm particles, 19% carbon loading, 100 Å pore size, from Waters Corp. The mobile phases ranged from 49 : 51 acetonitrile : water (v/v) to 100% acetonitrile for isocratic analyses, the flow rate of each mobile phase was 1.2 mL min^{-1} and the injection volume was 20 μL. For measurements of larger DNPH derivatives, the analytical column was one used for solanesol, the mobile phase was 95 : 5

acetonitrile : water (v/v) with a flow rate of 1.2 mL min^{-1}, and the injection volume was 20 μL.

GC-MS

The GC-MS system consisted of an Agilent 6890 GC interfaced to a 5973 MSD (mass selective detector). Analysis of oxidation products in cyclohexane solution was performed with a 15 m DB-5 Amine analytical column. The injection volume was 1 μL. The temperature program entailed an initial hold at 35 °C for 4 min, heating to 300 °C at 15 °C min^{-1}, and a final hold at 300 °C for 10 min. The mass spectrometer was operated in the electron-impact (EI) mode at 70 eV.

Recovery of solanesol from membrane filters

Filters were treated with 3 or 5 mL quantities of methanol by shaking for at least 30 s. Methanol solution in each methanol-quartz fiber filter mixture having the consistency of a pulp was recovered by means of a 10 mL syringe attached to PTFE tubing (9 cm × 3.2 mm id) equipped with a Luer lock at one end (part No. 90641, Hamilton Co., Reno, NV). Solutions were filtered with syringe filters containing PTFE membranes.

ES-MS

Analyses were performed with an LCQ-Duo Mass Spectrometer (Thermo-Finnigan) using electrospray ion generation. Samples were introduced *via* infusion at 25 μL min^{-1} using a 500 μL glass syringe and a syringe pump. For detection of solanesol in methanol, the solution was made alkaline with 0.50% ammonium hydroxide. For screening APE derivatives of solanesol oxidation products, scans initially were performed for *m/z* 100 to 1000, followed by scans of smaller portions of the mass spectrum. Finally, collision-induced fragmentation of individual ions was performed. One minute integration times were used. A default setting for ion lens voltages was used to provide adequate sensitivity across the mass range. All analyses were performed in the positive ion mode.

Stability of solanesol in the solid state

Pairs of quartz fiber membrane filters were fortified with 10 μg quantities of solanesol in 72 μL of methanol. One pair was stored for 6 days at room temperature in the dark and one pair was stored for 6 days at −10 °C. Then all filters were analyzed for solanesol content by HPLC.

Ozone monitoring

Three calibrated ozone monitors were employed: (1) a Model 49C UV Photometric Ozone Analyzer from Thermo Environmental Instruments Inc.; wavelength, 254 nm; range, 0–500 ppb; precision, 1 ppb, and (2) two Model 1003-RS UV Photometric Ozone Analyzers from Dasibi Environmental Corp.; wavelength, 254 nm; range, 0–500 ppb; precision, ±1% or 1 ppb, whichever is greater.

Ozone generation system for controlled atmospheres

The generation system was equipped with a Model XT-240 ozone generator from Air-Zone, Inc., Suffolk, VA, and was designed to produce ozone without nitrogen oxides. See p. S2 of the ESI.‡

Loading membrane filters approximately equally with tobacco smoke

A metal chamber sealed with a lid was filled with tobacco smoke from a lit Marlboro cigarette, and eight cassettes containing 37 mm membrane filters were placed into the metal chamber. Air sampling was conducted for 1 to 15 s. See p. S3 of the ESI.‡

Degradation of solanesol at low levels on filters during air sampling

Membrane filters (PTFE and quartz fiber) were fortified with 10 μg of solanesol in 72 μL of methanol solution. Also, eight quartz filters were loaded approx. equally with tobacco smoke; two of these filters were analyzed to determine the initial level of solanesol. Clean filters were fortified with pure solanesol at the initial level of solanesol found on the filters loaded with tobacco smoke. Air was drawn through the filters at 1.7 or 0.25 L min^{-1} for 1 to 24 h.

Large-scale degradation of solanesol during air sampling and identification of degradation products

Solanesol (37.4 mg, 5.9×10^{-5} mol) was dissolved in 16 mL of methanol in a midget bubbler. Purified air containing generated ozone at 38 ppm as a time-weighted average (TWA) was drawn through the bubbler inside a glove bag at 0.25 L min^{-1} for 2.9 h. Four S10 LpDNPH samplers were fortified with 150 μL aliquots of the solution of oxidation products with a syringe. After standing overnight, the DNPH derivatives in each sampler were eluted with 3 mL of acetonitrile. The eluates were combined, concentrated to 1.5 mL in an oven at 55 °C and analyzed by HPLC with standards at 360 nm. Additional methanol solution from the bubbler (8.5 mL) was blown down to 4.5 mL with a stream of pure air. APE (30 μL, 48 mg mL^{-1} in methanol) was added. Then the solution was diluted 1 : 20 with acetonitrile and analyzed by ES-MS.

In a second experiment, three quartz fiber filters were placed into each of 13 opaque filter cassettes in front of stainless steel support screens. Each set of filters was fortified with 3.5 mg of solanesol in 700 μL of methanol solution and dried in an oven at 65 °C. Environmental air containing ozone at 22.5 ppb (TWA) was drawn through each of the cassettes for 284 h at 0.2 L min^{-1} [relative humidity (RH) = ~60%]. Filters from each cassette were treated with 5 mL of methanol and methanol solutions were combined. Each of two S10 LpDNPH samplers was fortified with 150 μL of the methanol solution. DNPH derivatives from the samplers were recovered. The remaining methanol solution was filtered. APE (30 μL, 24 mg mL^{-1} in methanol) was added to a 2.25 mL aliquot of the methanolic filtrate. An aliquot was diluted 1 : 20 with solution A [consisting of 90 : 5 : 5 (v/v) acetonitrile : methanol : 0.01 M ammonium acetate adjusted to pH 6 with acetic acid] prior to ES-MS analysis.

In a third experiment, a single quartz filter was placed into each of 12 opaque filter cassettes and fortified with 3.5 mg of

solanesol in 100 μL of toluene. The filter cassettes were placed into a glove bag containing the ozone generator. Laboratory air, which had been filtered through coconut charcoal, was pumped into the glove bag (RH = ~50%). Air containing generated ozone at 0.5 ppm (TWA) was drawn through each filter cassette at 1 L min^{-1} for 1.5 h. Recovery from filters was accomplished with methanol. An S10 LpDNPH sampler was fortified with 150 μL of combined methanol solution. DNPH derivatives were recovered. The remaining methanol solution was filtered and blown down to 2.5 mL. APE (30 μL) was added, and the solution was diluted 1 : 100 with solution A.

In a fourth experiment, solanesol (75 mg; 1.2×10^{-4} mol) was dissolved in 16 mL of cyclohexane in a midget bubbler. Purified air containing ozone at 8.8 ppm (TWA) from an ozone generator was drawn through the bubbler inside a glove bag at 0.25 L min^{-1} for 7.5 h. The cyclohexane solution was reduced to 0.2 mL in an oven at 60 °C.

Passage of high-purity air through fortified filters

Dry, high-purity air inside a glove bag was drawn through six opaque filter cassettes containing quartz filters fortified with 10 μg of solanesol at 1.7 L min^{-1} for 1.6 h. The experiment was repeated with air at RH of 45%.

Test for organic nitrates

A pair of quartz fiber filters in an opaque cassette was fortified with 2.5 mg of solanesol in methanol solution. Environmental air was drawn through the quartz filters for 110 h at 1.7 L min^{-1} (total air volume = 11 m^3; ozone TWA = ~12 ppb; concentrations of environmental nitric oxide and nitrogen dioxide ranged from 1 to 67 ppb and from 7 to 41 ppb, respectively, as 1 h averages. Absorbing solution[39] was added to the quartz filters bearing degradation products. The test was negative with and without 16 h treatment of the sample at 92 °C in an oven (LOD of organic nitrates = <1 μg mL^{-1}).

Results and discussion

Measurement of solanesol by HPLC

HPLC analytical conditions similar to those in the ASTM method were employed for measurement of solanesol.[1,6] Variations from the ASTM method were (a) use of 100% acetonitrile as the mobile phase and (b) an increase in the flow rate of the mobile phase to 0.6 mL min^{-1}. Use of a small percentage of methanol in the mobile phase was found to be unnecessary; peak shapes and peak areas were virtually the same with and without 10% methanol in the mobile phase.

The limit of detection (LOD) and the lower limit of quantitation (LOQ) of solanesol in solution by HPLC were found to be 0.017 and 0.056 μg mL^{-1}, respectively. The LOD is defined as three times the standard error of the least squares calibration curve divided by the slope, and the LOQ is defined as ten times the standard error of the calibration curve divided by the slope.[40,41] Calibration curves for standards in the range of 0.12 to 10 μg mL^{-1} were linear with $R^2 = 0.9999$. Precision of measurement of solanesol was excellent; RSDs for eight injections of standards at 0.12 μg mL^{-1} and 3.0 μg mL^{-1} were 1.0% and 0.50%, respectively. Fig. 2 presents a chromatogram of tobacco smoke in methanol showing a prominent peak for solanesol (conc. of solanesol = 5.7 μg mL^{-1}).

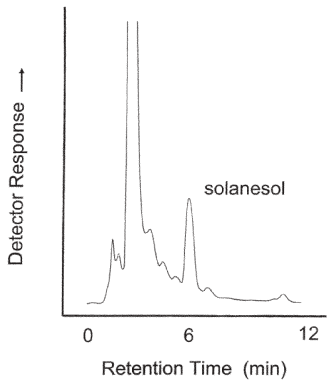


Fig. 2 Chromatogram of a solution of tobacco smoke in methanol (0.52 mg mL^{-1}) showing a peak for solanesol at a concentration of 5.7 μg mL^{-1}. Detector wavelength was 205 nm.

Stability of solanesol during storage

A 3 mL aliquot of solution of solanesol in methanol at 1.00 μg mL^{-1} was found to be stable with 100% recovery at room temperature in a tightly sealed, glass amber vial in the dark for at least 3.6 months. On the other hand, another aliquot of the same solution showed 6% deterioration (94% recovery) after storage in a tightly sealed, clear glass vial at room temp. under fluorescent lighting for 3.5 months.

Solanesol in the solid state degrades moderately rapidly at room temperature when exposed to environmental air under static conditions. Average recoveries of solanesol on filters stored for 6 days at room temp. in the dark and on filters stored for 6 days at −10 °C were 11% and 97%, respectively.

Solanesol content of tobacco smoke

The average quantity of solanesol in 2.99 to 3.43 mg of tobacco smoke on six PVC filters was determined to be 47.7 μg or 1.49% by weight. In comparison, solanesol generally makes up 2.3 to 3.2% of the mass of environmental tobacco smoke-respirable suspended particulate matter.[34]

Ratio of solanesol to total carbon in tobacco smoke

Total carbon (TC) was measured in tobacco smoke on 1.5 cm^2 punches from 37 mm quartz fiber filters by NIOSH method 5040.[4] Ratios for four filters ranged from 0.0218 to 0.0320 (solanesol : TC) for an average ratio of 0.0274. The inverse of the average ratio, which is 36.5, is comparable to the published average RSP : solanesol ratio of 38 : 1.[42]

Passage of purified air through solanesol-fortified filters

The average recoveries of solanesol from quartz filters after the passage of dry and humid, purified air through the filters were 98% and 99%, respectively. These results indicate that (a) solanesol is stable in dry, pure air in the dark, (b) humidity does not cause degradation of solanesol, and (c) the causative agent for degradation of solanesol must be a substance present in the environmental air.

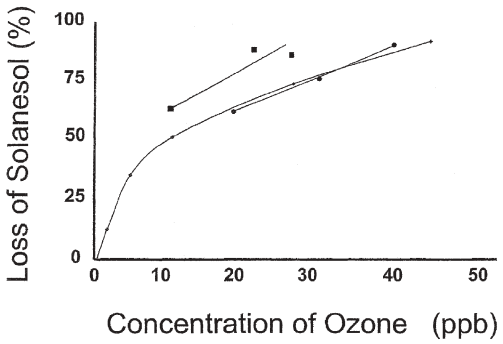


Fig. 3 Comparison of environmental air with ozone from an ozone generator. The diamonds and solid squares represent losses of solanesol from atmospheric ozone at relative humidity values of 50% ± 4%, and 29% ± 1%, respectively. The solid circles represent losses of solanesol from ozone which had been generated with the electric generator.

Comparison of ambient air with a generated ozone atmosphere for the degree of solanesol degradation

The three-point curve (solid circles) in Fig. 3 representing generated ozone at 56% RH matches very well with a segment of the curve representing atmospheric ozone (diamonds) at 50% RH, which was based on 1 h sampling periods at 1.7 L min^{-1} with glass fiber filters initially fortified with 10 μg quantities of solanesol. This excellent comparison coupled with the fact that solanesol on filters is stable during the passage of purified air through the filters is strong evidence that atmospheric ozone is indeed the oxidant in the atmosphere responsible for deterioration of solanesol during environmental air sampling. The solid squares in Fig. 3, based on 29% RH, demonstrate that solanesol is degraded to a greater degree at the lower RH at the sampling rate of 1.7 L min^{-1}.

Extents of degradation of solanesol as a function of sampling rate during environmental air sampling

The effect of sampling rates on degradation of solanesol was determined in two experiments in which quartz membrane filters had been fortified with 10 μg quantities of solanesol and environmental air sampling was conducted for 1 h (n = 5). In experiment 1, air containing ozone at 18.6 ppb as a TWA was sampled at 1.7 L min^{-1} (RH = 57%). Ozone (3.72 μg, 7.75 × 10^{-8} mol) in 102 L of air caused the oxidation of 70.4% of the solanesol (7.04 μg, 1.11 × 10^{-8} mol). Thus, the molar ratio of ozone to solanesol oxidized was 7.0 to 1. In experiment 2, air containing ozone at 13.4 ppb as a TWA was sampled at 0.25 L min^{-1} (RH = 90%). Ozone (0.395 μg, 8.23 × 10^{-9} mol) in 15 L of air caused oxidation of 30.0% of the solanesol (3.00 μg, 4.74 × 10^{-9} mol). Thus, the molar ratio of ozone to oxidized solanesol was 1.7 to 1 (or 1 to 1 if approx. 40% of the ozone passed through portions of the filters not bearing solanesol).

Two curves similar to the major curve shown in Fig. 3 which were based on 40% and 60% RH at a sampling rate of 0.25 L min^{-1} were constructed. The two curves (not shown), based on five different ozone levels, were virtually identical to each other, providing evidence that rates of degradation of solanesol are virtually independent of humidity at the lower sampling rate.

Degradation of solanesol in tobacco smoke by ozone

Air sampling using the ozone generation system was conducted at 0.25 L min^{-1} with smoke-loaded filters side-by-side with filters fortified with pure solanesol. Average recoveries of solanesol from smoke-loaded filters are presented in Table 1. While the first row pertains to glass fiber filters, all other rows in Table 1 pertain to the use of PTFE filters. Data in rows 2 and 3 show that a six-fold increase in the TWA concentration of ozone fails to increase the rate of degradation of solanesol at the same RH, while data in rows 4 and 5 show that the effect of a six-fold increase in ozone concentration on solanesol might be marginal. Data not presented in Table 1 show that (a) solanesol as a pure compound on a filter is degraded much more rapidly than solanesol as a component of tobacco smoke on a filter, (b) the extent of degradation of pure solanesol on a filter can be as much as 100%, and (c) pure solanesol is degraded on PTFE membrane filters more rapidly than pure solanesol on quartz fiber membrane filters during air sampling.

ES-MS confirmation of solanesol loss during air sampling

Typically in ES-MS, the protonated $[M + H]^+$ ion of a compound is observed in positive ion mode (for solanesol, this would be at m/z 631). Solanesol does not protonate to a significant degree but efficiently forms a positively charged adduct with ammonium ion in 100% methanol (the adduct formation is highly solvent-dependent). Ammonium impurities in the solvent produced measurable signal for solanesol standards; addition of 0.50% ammonium hydroxide (v/v) significantly enhanced this signal. The main ion signal was observed at m/z 648 with smaller signals visible at m/z 630 (due to dehydration) and 613 (presently unidentified). These probably were generated because the ES-MS instrument uses passage through a heated capillary (200 °C) to eliminate solvent from ions prior to detection (see Fig. 4). Substitution of ^{15}N ammonium hydroxide shifted the main ion signal to m/z

Table 1 Degradation of solanesol in tobacco smoke by ozone

Ozone TWA (ppb)	Relative humidity (%)	Sampling period/h	Initial quantity of solanesol/μg	Average recovery of solanesol after ozone treatment (%)
29	25	4.4	24	61
18	43	1.3	2.6	80
108	43	1.3	2.6	82
18	25	3.0	6.4	71
114	25	3.0	6.4	61

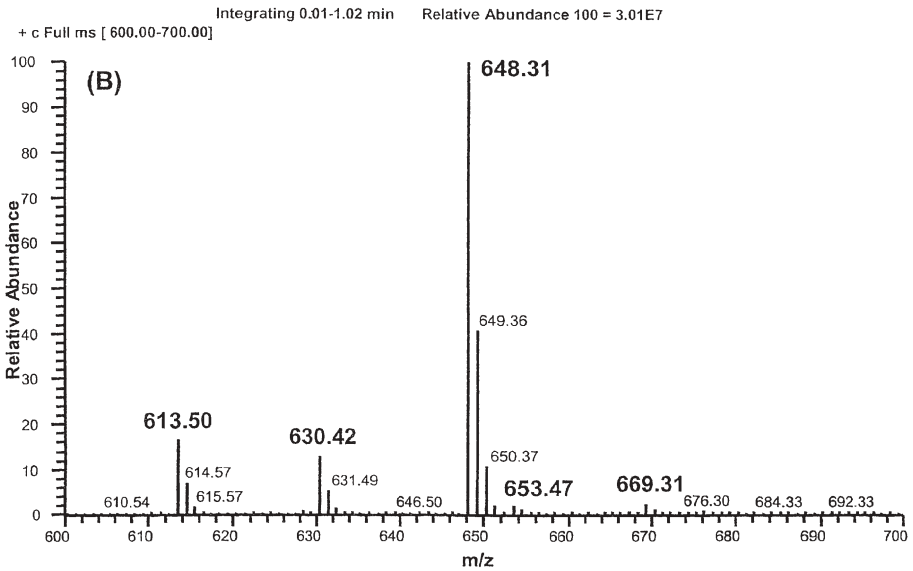


Fig. 4 Solanesol (1.0 μg mL^{-1}) observed as the ammonium adduct at *m/z* 648 in ES-MS using 99.5 : 0.5 methanol : ammonium hydroxide (v/v), delivered *via* infusion at 25 μL min^{-1}. Note the low-intensity signals for the Na^+ and K^+ adducts at *m/z* 653.47 and 669.31, respectively.

649, confirming ammonium adduct formation. Collision-induced dissociation of the solanesol–ammonium adduct produced a characteristic fragment ion spectrum with the most intense ion at *m/z* 630, permitting confirmation of solanesol.

A 1 μg mL^{-1} solanesol standard prepared in alkaline methanol and analyzed gave a strong signal for the solanesol–ammonium adduct. By use of syringe infusion at 25 μL min^{-1}, a scanning range of *m/z* 600–660 and instrument parameters tuned to maximize the solanesol–ammonium adduct signal, the LOD of solanesol was estimated at 0.01 μg mL^{-1} by ES-MS. ES-MS did corroborate HPLC in confirming that losses of solanesol occurred during environmental air sampling.

ES-MS screening of solanesol oxidation products in methanol (bubbler)

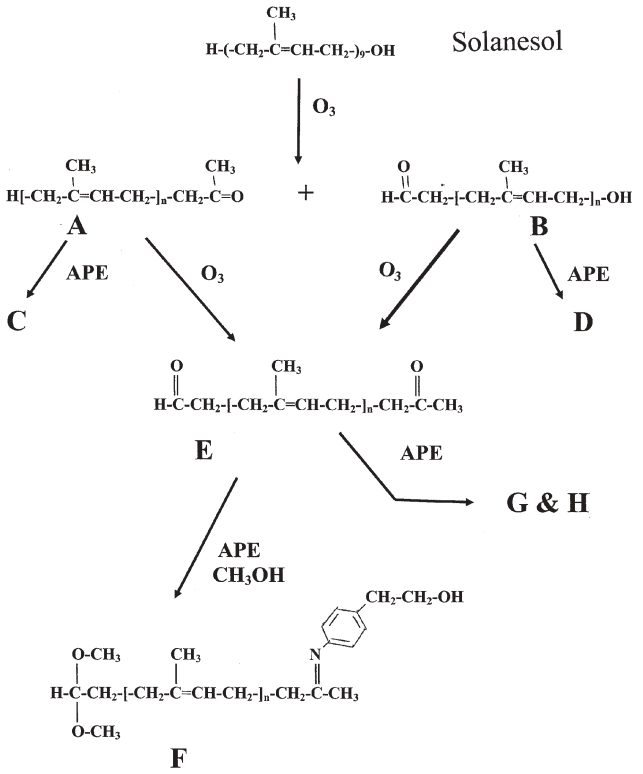
Although very weak $[M + H]^+$ ion signals for various ozonation products of solanesol could be observed in acidic media at sufficiently high concentration, considerably more intense signals were obtained for imine derivatives of these products with APE, which provides both easily protonated nitrogen and oxygen atoms. The APE derivatives proved most stable in 100% acetonitrile. Although ion signals were only moderately intense, it was not possible to enhance ionization by using high percentages of protic solvents, such as methanol, or by adding acid, which quickly destroyed the APE derivatives. Small percentages of protic solvents and buffers appeared to be acceptable, and even seemed to enhance the ion signals observed for low levels of APE derivatives (see the aforementioned Solution A in Experimental section). Acetonitrile also provided a serendipitous benefit; unlike 100% methanol, acetonitrile did not promote formation of the solanesol–ammonium adduct. Thus, even if a large quantity of solanesol remained in the ozonation mixture, the solanesol did not produce a significant ion signal, which could suppress ion signals for the APE derivatives.

Ion signal intensities for the APE derivatives of isoprenoid acetone standards (Table 2) were sufficient for our purposes. The fragment ion spectra obtained *via* collision-induced dissociation were consistent with the structures of APE derivatives of isoprenoid acetones. Reliable patterns for the fragment ions were apparent; *e.g.*, the most intense fragment was always *m/z* 190. This allowed us to predict reliably fragment ions expected for isoprenoid acetones for which standards were not available, such as farnesylgeranylgeranylacetone.

The structure of solanesol with its nine isoprene units implied that its oxidation products would follow predictable molecular mass patterns (68 *m/z* apart). Close examination of the mass spectrum revealed seven groups of products of this type (see Fig. S2 and S3 in the ESI‡). Three of these groups are consistent with oxidation of double bonds of solanesol by ozone (see series A, B, and E in Scheme 1). One of the groups appears to be the dimethyl acetals of product series E, resulting from the reaction taking place in methanol solution (see product series F in Scheme 1). Another group is consistent with loss of methanol from these dimethyl acetals to form

Table 2 Structures and common names of the isoprenoid acetones

General structure (all *E*; all *trans*)	$H\text{-}[\text{-}CH_2\text{-}C(CH_3)=CH\text{-}CH_2\text{-}]_n\text{-}CH_2\text{-}C(CH_3)=O$
Isoprenoid acetone	*n*
Acetone	0
6-Methyl-5-hepten-2-one	1
Geranylacetone	2
Farnesylacetone	3
Tetraprenylacetone	4
Geranylfarnesylacetone	5
Farnesylfarnesylacetone	6
Farnesylgeranylgeranyl-acetone	7
Bombiprenone	8



Scheme 1 Pathways for formation of three series of ozonation products from solanesol. Products in series A, B, and E are isoprenoid acetones, ω-hydroxyisoprenoid acetaldehydes and isoprenoid oxoaldehydes, respectively. Products in series A, B, and E can react with methanol in the midget bubbler to form dimethyl acetals. Dimethyl acetals as their APE derivatives are not observed by ES-MS after ozonation products on quartz fiber filters are treated with methanol. Instead, products in series E on quartz filters form mono APE and bis (APE) derivatives upon addition of APE (series G + H).

methoxyvinyl species, probably occurring in the heated capillary or mass spectrometer (see Fig. 5 for a general structure). We have not been able to draw any conclusions about the nature of the other two groups.

APE derivatives of all of the isoprenoid acetones expected from the ozonation of solanesol were observed in the mixture from the bubbler (see product series C in Scheme 1 and list in Table 2). For lower members of the series, fragment ion spectra matched those observed for APE derivatives of corresponding standards; for higher members, the fragments were as expected *via* extrapolation.

Many of the other ions observed in the mass spectrum were consistent with APE derivatives of ω-hydroxyisoprenoid acetaldehydes and APE derivatives of dimethyl acetals of isoprenoid oxoaldehydes (see product series D and F in Scheme 1). Since the solanesol ozonation was performed in 100% methanol, formation of dimethyl acetals would be

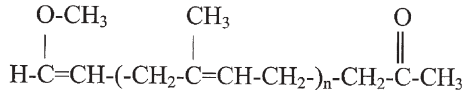


Fig. 5 General structure of a methoxyvinyl derivative of an isoprenoid oxoaldehyde resulting from loss of methanol from a dimethylacetal of an oxoaldehyde.

expected. Due to the unavailability of standards for isoprenoid oxoaldehydes and ω-hydroxyisoprenoid acetaldehydes, ES-MS response for the anticipated APE derivatives could not be explored as had been done with the isoprenoid acetones. However, the molecular masses of observed protonated ions and the fragment spectra obtained *via* collision-induced dissociation are consistent with the expected products.

Thus, Scheme 1 presents pathways for formation of products by ozonation of solanesol in excess, a scheme which is consistent with the ions observed by ES-MS. Ozonation of solanesol in methanol to 25% to 60% completion gave rise to three series of ozonation products: (1) isoprenoid acetones with acetone as the simplest product (product series A with $n = 0$ to 8, Table 2), (2) ω-hydroxyisoprenoid acetaldehydes with α-hydroxyacetaldehyde as the simplest product (product series B with $n = 0$ to 8), and (3) isoprenoid oxoaldehydes with 4-oxopentanal as the simplest product (product series E with $n = 0$ to 7). The reaction pathway in Scheme 1 is limited by the fact that ozone reacts most rapidly with carbon–carbon double bonds.[43] In addition, acetone, α-hydroxyacetaldehyde and 4-oxopentanal react very slowly with ozone,[43] and product series A, B and E account for 26 ozonation products. All ozonation products in series A and B were observed to react with APE to form APE derivatives (product series C and D, respectively). All ozonation products in series E were observed to react with methanol during ozonation in the bubbler to form the corresponding dimethyl acetals. The dimethyl acetals, in turn, react with APE to form the corresponding APE derivatives (product series F).

The ES-MS data for the bubbler sample also indicated ion signals for other APE derivatives which were derived from solanesol as shown by 68 *m*/*z* unit separation (see Table S1 in the ESI‡). It is assumed that these are artifact signals generated from the APE derivatives of the main solanesol oxidation product series during analysis. For example, a degree of unintended ion fragmentation was found to occur during passage of ions through the heated capillary (200 °C) used for desolvation. Tests demonstrated that the relative intensities of different sets of APE derivative ions were dependent on desolvation temperature.

ES-MS identification of oxidation products on quartz filters after environmental air sampling

Environmental air sampling with solanesol-fortified quartz fiber filters for 284 h gave rise to oxidation of an average of 28% (977 μg) of the original 3.5 mg of solanesol per filter cassette (13 cassettes total). ES-MS showed the presence of 24 of the 26 oxidation products expected in Scheme 1 for ozone as the oxidant of solanesol (see Fig. 6A). However, unlike the dimethyl acetals formed in methanol solution in a bubbler, the mono APE derivatives and bis (APE) derivatives of isoprenoid oxoaldehydes themselves (with no observed dimethyl acetal formation) were observed for products found on the quartz filters.

ES-MS identification of oxidation products after air sampling in an atmosphere containing generated ozone

Air sampling with solanesol-fortified quartz fiber filters inside a glove bag containing generated ozone for 1.5 h gave rise to

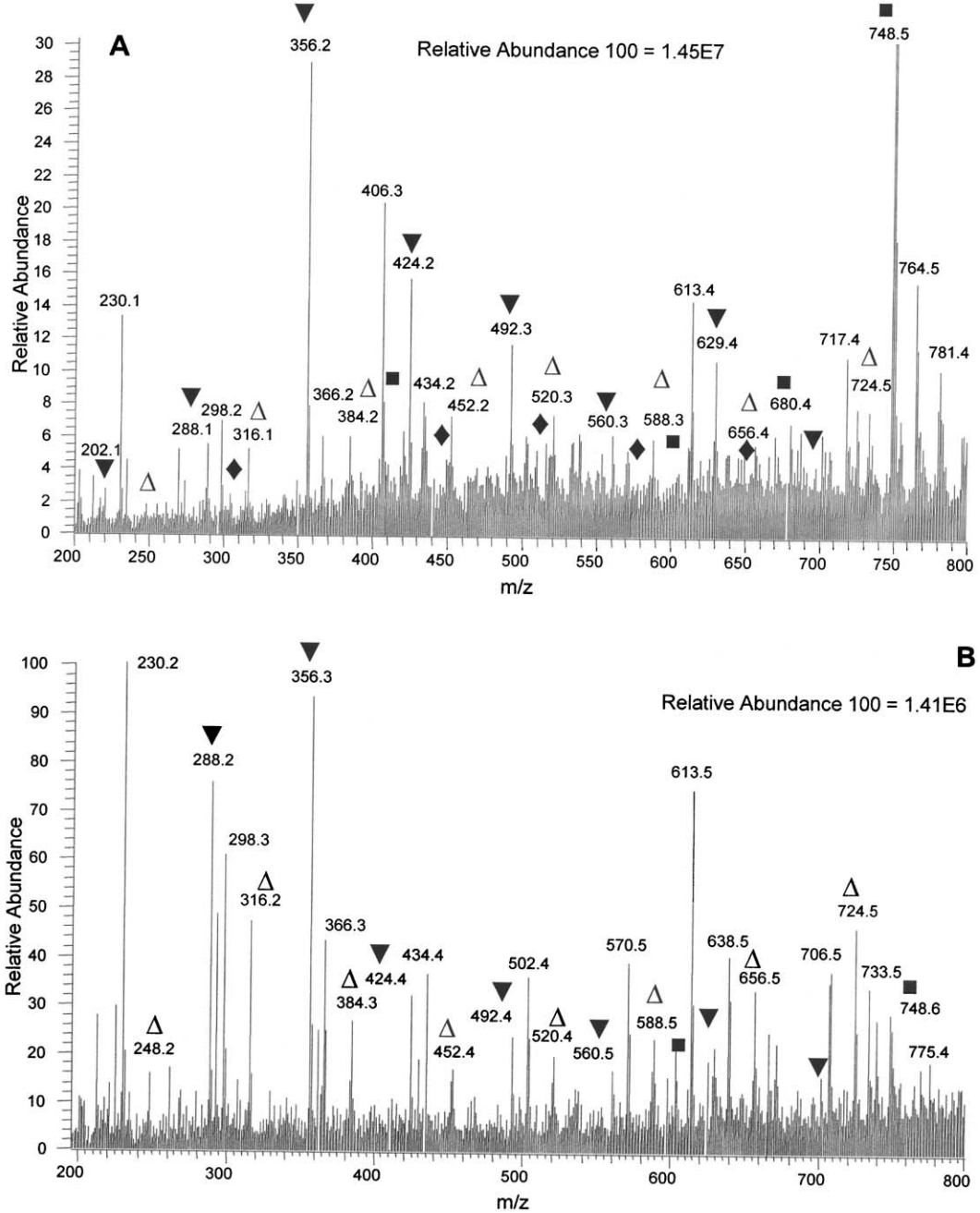


Fig. 6 ES-MS analysis of ozonation products of solanesol on quartz fiber filters following environmental air sampling (A) and air sampling with air containing generated ozone at 0.5 ppm (B) after derivatization with 2-(*p*-aminophenyl)ethanol and 1 : 20 dilution with a solution of 90 : 5 : 5 (v/v) acetonitrile : methanol : 0.01 *M* ammonium acetate adjusted to pH 6 with acetic acid. Sample was infused at 25 μL min^{-1} (1 min integration, MS default settings). Symbols: Open triangles (△), solid triangles (▼), solid squares (■), and diamonds (♦) indicate APE derivatives of ω-hydroxy-isoprenoid acetaldehydes, mono APE derivatives of isoprenoid oxoaldehydes, bis (APE) derivatives of isoprenoid oxoaldehydes, and APE derivatives of isoprenoid acetones, respectively.

an oxidation of an average of 22% (775 μg) of the original 3.5 mg of solanesol per filter cassette (12 cassettes total). The ES-MS spectrum indicated the presence of (a) the APE derivatives of all nine ω-hydroxyisoprenoid acetaldehydes (product series D; the ion for α-hydroxyacetaldehyde was very weak but the presence of the other eight products was obvious), (b) the mono APE derivatives of all eight isoprenoid oxoaldehydes (product series G; ions for $n = 0$ and 7 were very weak, ions for $n = 1$ and 6 were moderate and all other ions were strong), and (c) the bis (APE) derivatives of a few of the isoprenoid oxoaldehydes (product series H); the product series $n = 6$ obviously was present and the other ions were either very weak or not observed) (see Fig. 6B). One other group of ion signals at 68 *m*/*z* unit intervals was observed for these filter samples, which corresponded to a set of APE derivatives that was observed for the bubbler sample and

was attributed to the generation of artifact ions during ES-MS analysis.

The intensities of ion signals of solanesol oxidation products and related instrumental artifacts varied greatly from one ozonation experiment to another. Generally, the bubbler experiment gave the greatest yield of fairly intense ion signals while solanesol-fortified filters subjected to environmental air draw and ozone-enhanced air draw yielded progressively fewer intense signals (compare Fig. S2, S4 and S5 in the ESI‡). This was especially notable for the isoprenoid acetones: all but the bombiprenone derivative were clearly visible for the bubbler experiment, but signals were far weaker for filters/environmental air and virtually undetectable above background noise for filters/ozone-enhanced air. Several reasons can be suggested for this variable response: (1) bubbler and filter experiments were conducted several months apart, allowing drift in instrument performance, (2) the ratio of quantity of APE added to the quantity of isoprenoid acetones present varied with analyte concentration, altering reaction equilibrium for derivative formation, (3) different degrees of sample dilution were performed prior to ES-MS analysis (since concomitant species lead to ion signal suppression in ES-MS, the extent of dilution strongly impacts signal intensities), and (4) variability in ozonation conditions including concentration of ozone in air, sampling rate, sampling period, relative percentages of solanesol converted, and state of solanesol (solution in bubbler or solid on filters).

Examination of the two mass spectra in Fig. 6 indicate that environmental air sampling with solanesol-fortified filters and air sampling with solanesol-fortified filters in an atmosphere containing generated ozone give rise to many of the same oxidation products. This comparison is additional strong evidence that ozone is indeed the agent in the atmosphere responsible for degradation of solanesol during environmental air sampling.

Oxides of nitrogen are ruled out as major oxidants of solanesol during environmental air sampling because the sensitive test for organic nitrates was negative.[39] Photodecompostion of solanesol is ruled out because all air sampling experiments were conducted with fortified filters inside opaque filter cassettes to prevent the transmission of light.

HPLC detection of oxidation products in bubbler and on quartz filters after air sampling

Ten significant oxidation products of solanesol were detected routinely as their DNPH derivatives, 4-oxopentanal and nine isoprenoid acetones (Table 2) in the ozonation mixtures both (a) in methanol solution from the midget bubbler and (b) on solanesol-fortified quartz fiber filters following air sampling. Standards of DNPH derivatives of all of the isoprenoid acetones were available for determination of retention times with the exception of farnesylgeranylgeranylacetone and bombiprenone. A chromatogram of the DNPH derivatives of the larger isoprenoid acetones is shown in Fig. 7.

After environmental air sampling with solanesol-fortified quartz fiber filters, acetone and all other isoprenoid acetones except 6-methyl-5-hepten-2-one were detected as their DNPH derivatives by HPLC. The lack of detection of

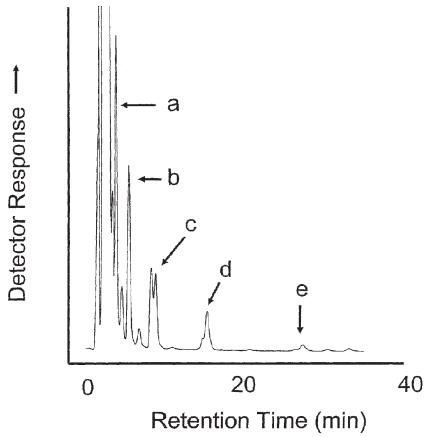


Fig. 7 HPLC chromatogram of DNPH derivatives of the larger isoprenoid acetones from the ozonated mixture in methanol. Identification of peaks: (a) tetraprenylacetone–DNPH, (b) geranylfarnesylacetone–DNPH, and (c) farnesylfarnesylacetone–DNPH. Peaks at "d" and "e" may belong to farnesylgeranylgeranylacetone–DNPH and bombiprenone–DNPH, respectively.

6-methyl-5-hepten-2-one from quartz filters apparently was due to virtually complete evaporation during the sampling periods. However, acetone, which is more volatile than 6-methyl-5-hepten-2-one, was always detected as its DNPH derivative by HPLC after environmental air sampling with quartz fiber filters. Reasons are that some organic vapors are known to be adsorbed by quartz fiber filters during environmental air sampling[44] and acetone is a common environmental air pollutant. Thus, one should compare sample filters with blank filters.

In addition to acetone as its DNPH derivative, 2-butanone as its DNPH derivative was always found on quartz fiber filters during environmental air sampling. 2-Butanone is another environmental air pollutant. However, since 2-butanone was found in all ozonation experiments with solanesol involving bubblers and filters, it appears that 2-butanone may be a minor ozonation product of solanesol.

Although formaldehyde was detected in bubblers, it appeared that all formaldehyde formed was produced by oxidation of solvent by ozone.

GC-MS analysis of ozone oxidation products from solanesol

Fig. 8 presents a total ion chromatogram (TIC) of a concentrated solution of ozonation products of solanesol following ozonation of a cyclohexane solution of solanesol in a bubbler. The TIC indicates the presence of a very complex mixture. Peaks for most of the expected isoprenoid acetones are observed in this TIC (see Table 2). All of these isoprenoid acetones show very similar fragmentation patterns in the mass spectra as would be expected. The use of cyclohexane as solvent is an advantage over the use of methanol in this experiment in that most or all ozonation products of solanesol have a greater solubility in cyclohexane. Consequently, a much greater fraction of the ozonation products remain in solution during the evaporation of cyclohexane (for concentration) and much larger peaks are observed in the TIC. Acetone (if present) co-eluted with the solvent and was not observed.

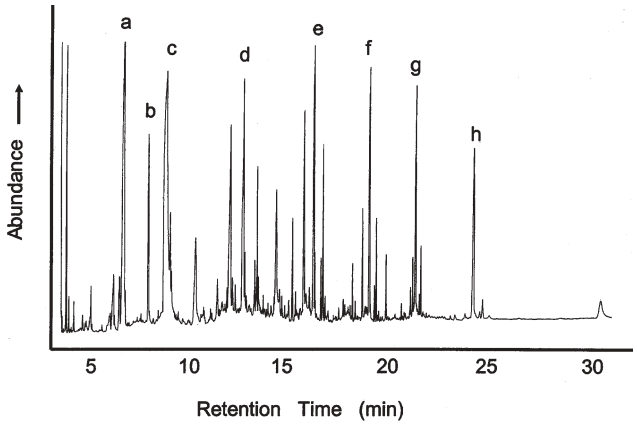


Fig. 8 Total ion chromatogram from GC-MS of ozonated solution of solanesol in cyclohexane. Identification of peaks: (a) cyclohexanol and cyclohexanone (impurities in solvent), (b) 6-methyl-5-hepten-2-one, (c) unidentified peak, MW $\geqslant$ 116, (d) geranylacetone, (e) farnesylacetone, (f) tetraprenylacetone, (g) geranylfarnesylacetone, and (h) farnesylfarnesylacetone.

Comparison of indoor ozone levels with outdoor ozone levels

In spite of indoor ozone surveys, misconceptions continue that significant ozone levels exist chiefly outdoors and that indoor levels are inconsequential.[45] The National Ambient Air Quality Standard (NAAQS) for ozone is 120 ppbv, and many areas of the U.S. have failed to meet this standard. Typically, indoor ozone levels are 20 to 80% of the outdoor ozone levels, depending on the ventilation rate.[45] Decay rates of ozone are significant when ventilation rates drop to values near zero. Because of the high rate of decay of indoor ozone when ventilation rates are very low, typical indoor ozone levels have been found to be less than 10% of outdoor levels when air conditioning is in use.[46] Ground level ozone levels can exceed the NAAQS by 25% in rural areas.[47] Thus, serious ozone levels can exist in wide areas of the U.S. and are not limited to urban areas, contrary to what many people might believe. Therefore, solanesol can be an unreliable quantitative marker for tobacco smoke during indoor air sampling when indoor ozone levels are greater than about 15 ppb. Ozone probably exists in mines when miners are present because fresh air from the outdoors is pumped into the mines.

An approach to avoiding solanesol degradation by ozone

One approach to solving the problem of degradation of solanesol on the membrane filter during air sampling is to precede the air sampler with an ozone scrubber, such as a denuder coated with potassium iodide. Ozone in the air stream can diffuse to the walls of the denuder where it is destroyed through reaction with potassium iodide, while the solanesol-containing particles can pass through the denuder for collection on the filter. D. Helmig presents an in-depth discussion of ozone removal techniques during air sampling, including the use of potassium iodide denuders.[37]

However, an ideal ozone scrubber would appear to have its shortcoming in the solanesol air sampling method because ozone in the air at points distant from the scrubber–sampler combination will be degrading solanesol in tobacco smoke. Thus, one question would deal with what fraction of the solanesol is degraded by ozone in air which has not reached the scrubber. The answer to this question is not immediately obvious and may depend on sampling rate, sampling period and the concentration of tobacco smoke in the air, a concentration which might tend to be highly variable.

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