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Note

γ-Radiolysis of acetylsalicylic acid in the solid-state gas chromatographic–mass spectrometric and high-performance liquid chromatographic identification and quantification of the radiolytic products

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γ-Irradiation is commonly used for the sterilization of drug substances for injectable preparations. Whereas the effects of γ-rays on aqueous solutions of salicylates have been subject of several publications, relatively little work has been carried out on powders.

Downes[1] studied alkaline salicylate solutions and noted the formation of phenol and 2,3- and 2,5-dihydroxybenzoic acids. Loeff and Swallow[2] reported, in a reference to unpublished work, the formation of 2,3-, 2,4- and 2,5-dihydroxybenzoic acid from salicylic acid. Other studies indicate decarboxylation in deaerated, neutral solutions leading to the formation of dihydroxybiphenyls with no detectable hydroxylation[3]. In both acidic and alkaline solutions the formation of catechol has also been reported[3,4].

The solid-state radiolysis of salicylates has not been studied in detail. De la Paz[5] studied irradiation in the solid phase of salicylamide, phenyl salicylate and acetylsalicylic acid and almost no degradation was observed at doses as high as 4.5 Mrad.

This work was undertaken in order to obtain more quantitative information on the extent and nature of the degradation of acetylsalycylic acid in the solid state by sterilizing radiation, particularly γ-rays.

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EXPERIMENTAL

Chemicals and reagents

All chemicals were of the highest purity available. Acetylsalicylic acid was obtained from Rhône Poulenc Santé (La Défense, France). Solvents used for high-performance liquid chromatography (HPLC) were of HPLC grade. *o*-Hydroxyacetophenone was purchased from Ega-Chimie (Strasbourg, France). Phenyl acetate was synthesized as follows: phenol and acetic anhydride were mixed in a 1:1 molar ratio and dissolved in dichloromethane. After addition of several drops of concentrated sulphuric acid, the mixture was refluxed for 2 h. After neutralization with 1 *M* sodium hydroxide solution, the organic phase was dried over sodium sulphate and distilled.

High-performance liquid chromatography

The HPLC apparatus consisted of WISP 710B automatic sample injector (Millipore, Waters Chromatography Division, St. Quentin en Yvelines, France), a ConstaMetric III G pump (LDC, Paris, France), a Beckman Model 165 rapid scanning detector (Beckman Instruments France, Gagny, France) and a Spectra-Physics Model SP 4200 electronic integrator (Spectra-Physics France, Les Ulis, France). The chromatographic column was a Perkin-Elmer HS-5 C18 (125 × 4.6 mm I.D.) (Perkin-Elmer, Bois d'Arcy, France).

The mobile phase was acetonitrile–methanol–5 m*M* NaH_2PO_4 (pH 2.35) (10:10:80, v/v). The flow-rate was 2.0 ml/min and the temperature was ambient. The detection wavelength was 246 nm (0.02 a.u.f.s.).

The working solutions of acetylsalicylic acid were prepared at a concentration of 20% (w/v) in methanol–water (1:4, v/v).

The chromatographic peaks were quantified by external calibration using electronically integrated peak areas.

Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were performed using a VG-Micromass 7035 double-focusing mass spectrometer equipped with a Dany 3800 gas chromatograph (VG Analytical, Manchester, U.K.). The capillary column was made of fused silica (25 m × 0.22 mm I.D.) with a chemically bonded CP-Sil 5 CB stationary phase (Chrompack, Les Ulis, France) maintained at 280°C. The carrier gas was helium (N55) and the inlet pressure was 0.90 bar. The split/splitless injector was in the split position (1:20) and its temperature was 250°C. The temperature gradient was from 25 to 250°C at 5°C/min. The mass spectrometer was operated under the following conditions: electron energy, 70 eV; ion source temperature, 200°C; resolution 1000.

Preparation of samples

Irradiation of acetylsalicylic acid. Samples were irradiated using a cobalt-60 γ-ray source. The dose was calculated using Red or Clear Perpex dosimeters (Harwell, U.K.). All irradiations were carried out at room temperature.

Extraction. A 50-g sample of acetylsalicylic acid was dissolved in 150 ml of distilled water and the pH was adjusted to 7.5 with aqueous ammonia. The solution was extracted twice with 100 ml of hexane and the organic phase was dried with magnesium sulphate, filtered through deactivated glass-wool and evaporated to 0.5 ml under a stream of helium. A 2-μl sample was used for the GC–MS analyses.

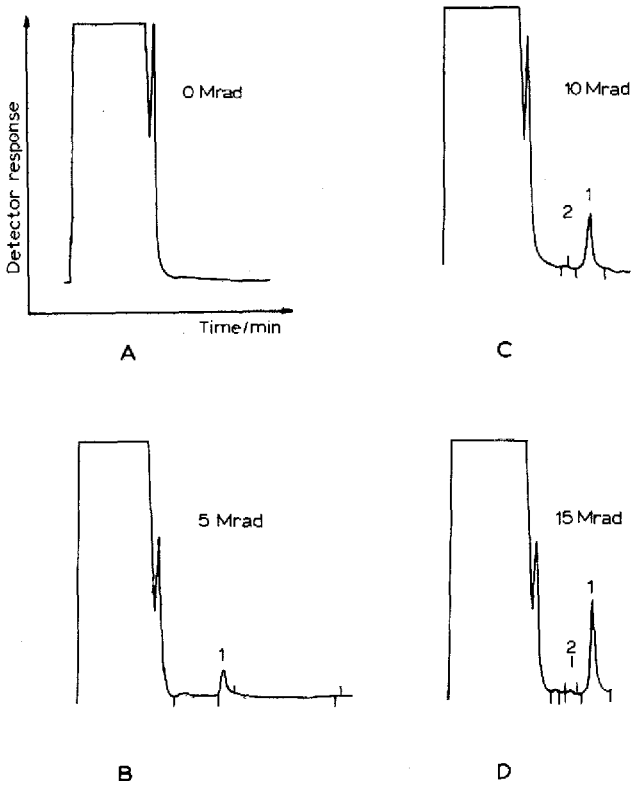


Fig. 1. HPLC traces of acetylsalicylic acid: (A) reference non-irradiated; (B) irradiated at a dose of 5 Mrad; (C) irradiated at a dose of 10 Mrad; (D) irradiated at a dose of 15 Mrad. The volume injected corresponded to 10 mg of acetylsalicylic acid in each instance. Peaks: 1 = *o*-hydroxyacetophenone; 2 = phenyl acetate.

RESULTS AND DISCUSSION

HPLC analysis

Radiolytic degradation products of acetylsalicylic acid were analysed directly by HPLC with UV detection at 246 nm. The chromatograms of the reference sample of non-irradiated acetylsalicylic acid and those irradiated at doses of 5, 10 and 15

TABLE I

QUANTITATIVE HPLC ANALYSIS OF SAMPLES OF γ-IRRADIATED ACETYLSALICYLIC ACID

Dose (Mrad)	*1 (ppm)*	*2 (ppm)*
0	0	0
5	19	Not detected
10	42	19
15	62	51

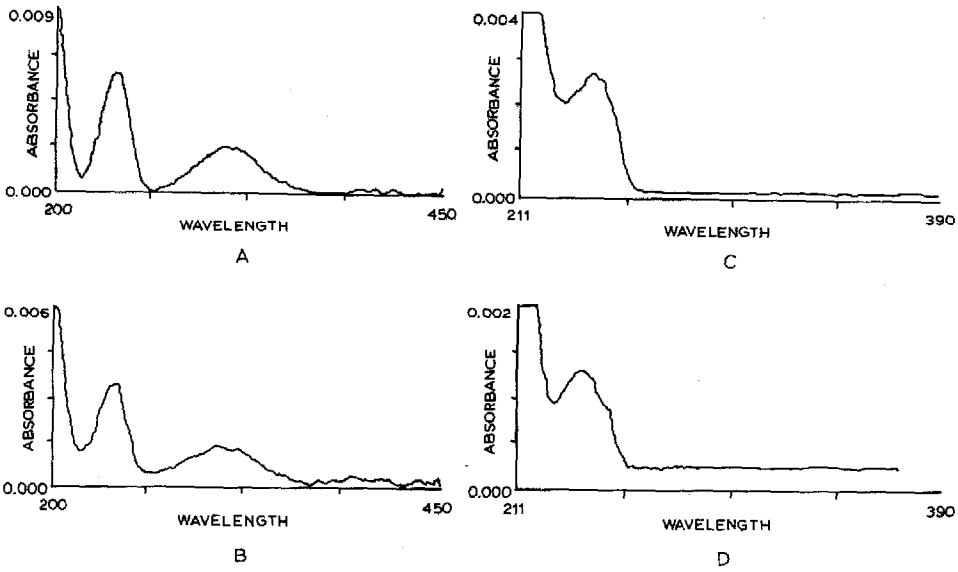
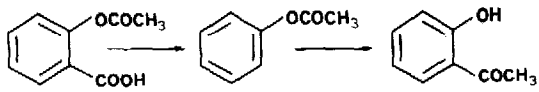


Fig. 2. UV spectra of (A) peak 1 in Fig. 1; (B) *o*-hydroxyacetophenone reference compound; (C) peak 2 in Fig. 1; (D) phenyl acetate reference compound. The spectra were obtained on-line with the HPLC separation.

Mrad are shown in Fig. 1. The two major products formed in the course of γ-irradiation were found to increase with increasing radiation dose (Table I). Their UV spectra, determined on-line with the HPLC separation, corresponded to those of phenyl acetate (2) and *o*-hydroxyacetophenone (1). Fig. 2 shows the spectra of the two major degradation products 1 and 2 (Scheme 1) and those of the corresponding reference compounds.



Scheme 1. (2) (1)

In order to characterize these compounds further, we have tried to isolate them by preparative HPLC. However, these attempts failed owing to the relatively high volatility of the compounds investigated.

GC–MS analysis

Prior to the GC–MS analyses, samples of acetylsalicylic acid were extracted with hexane using the procedure described under Experimental. The chromatograms of the samples irradiated at three dosages were similar. A typical example of the hexane extract is shown in Fig. 3. Approximately twenty compounds were detected. The two major compounds were *o*-hydroxyacetophenone (1) and phenyl acetate (2). Their structures were confirmed by comparison of their GC retention behaviour and mass spectra (Fig. 4A) with those of the authentic compounds.

In addition to the major degradation products (1 and 2), the structures of six

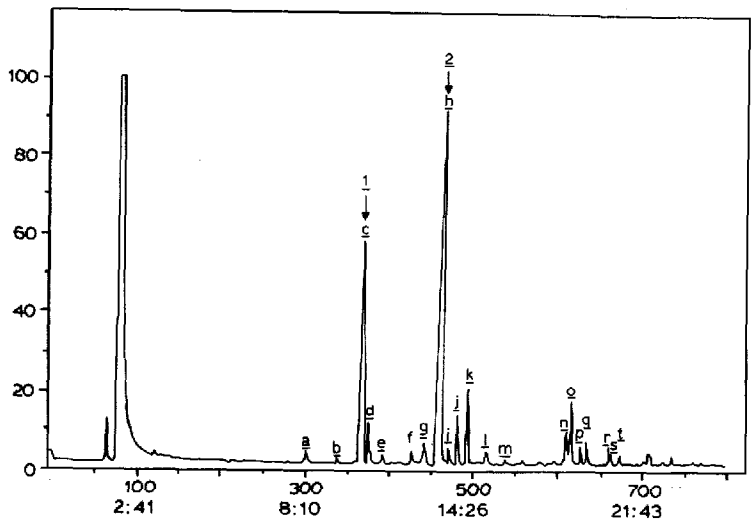


Fig. 3. Chromatogram of the hexane extract of a sample of acetylsalicylic acid irradiated at a dose of 15 Mrad and analysed by GC–MS.

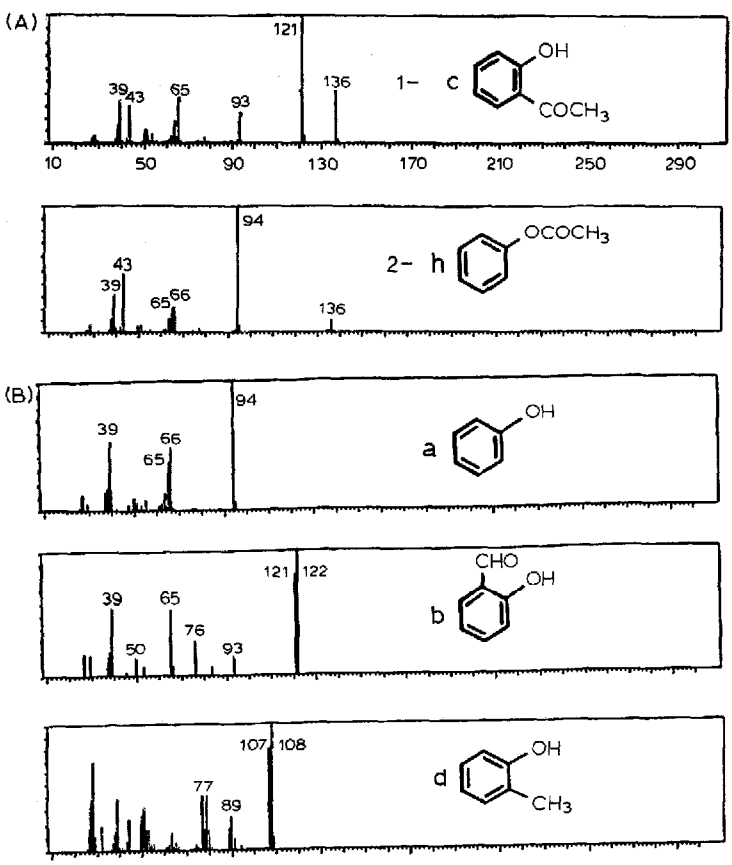


Fig. 4.

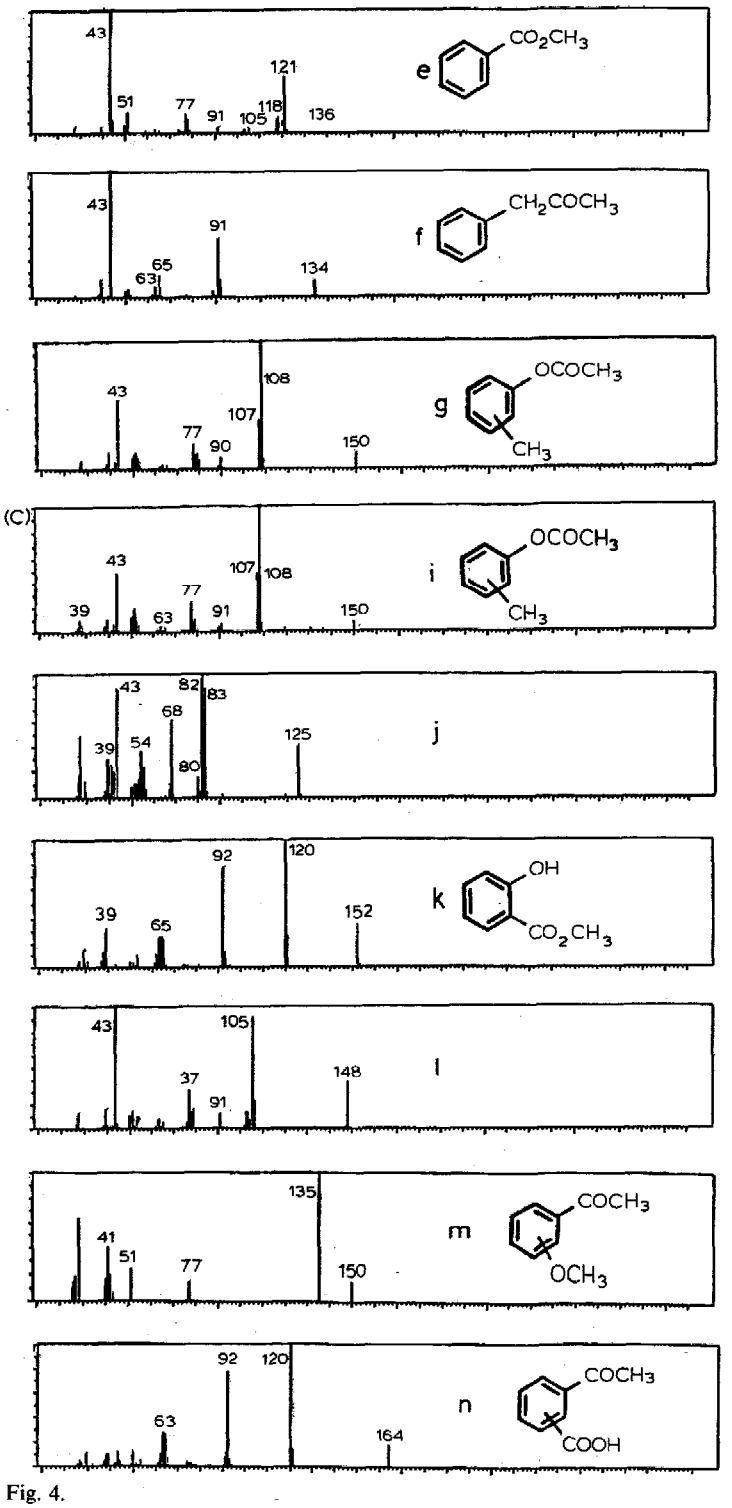


Fig. 4.

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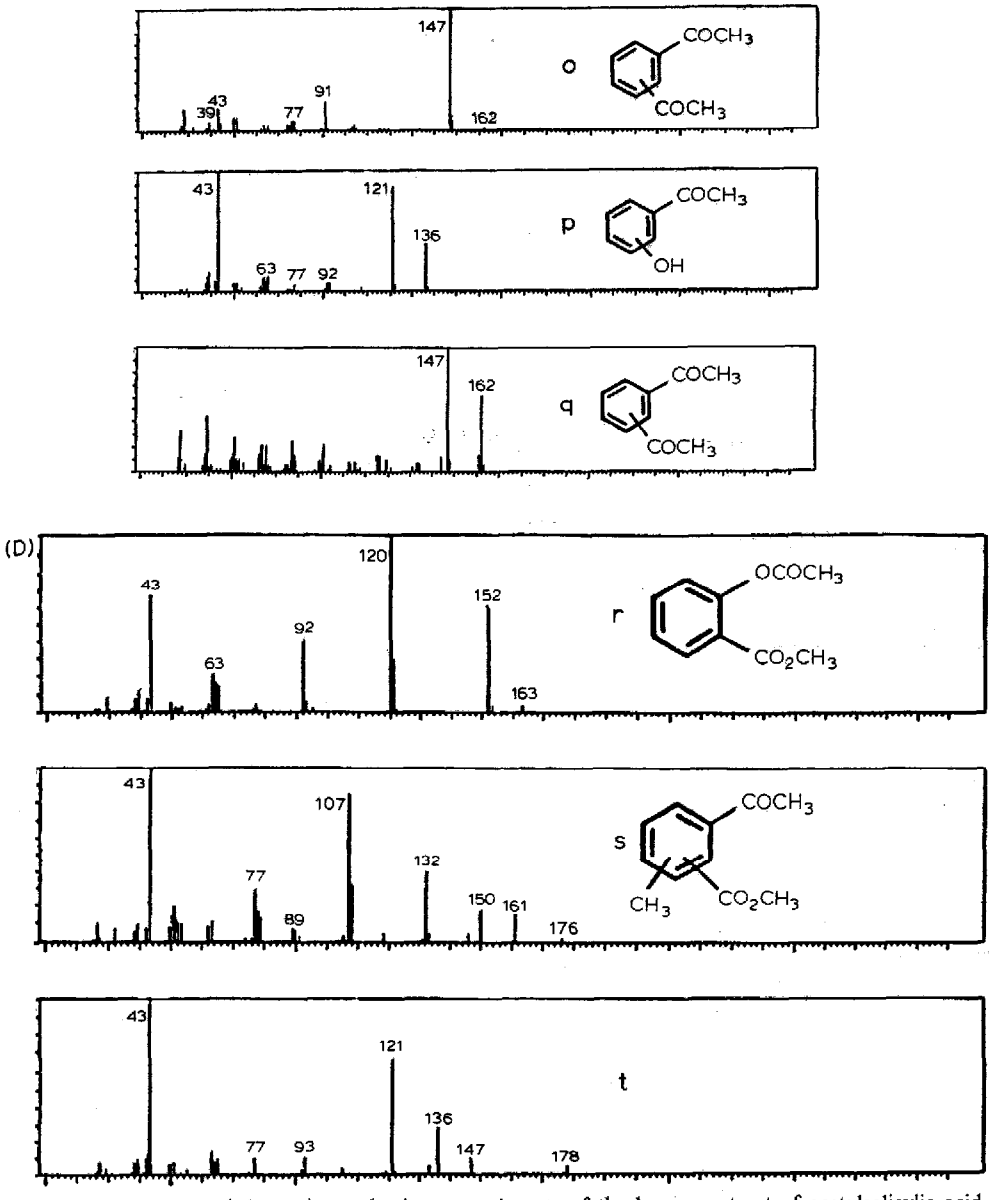

Fig. 4. Mass spectra of the major and minor constituents of the hexane extract of acetylsalicylic acid irradiated at a dose of 15 Mrad.

other compounds were determined unambiguously (a, b, d, e, f and k), while those of nine compounds were proposed on the basis of a single mass spectrum (Fig. 4B–D).

CONCLUSIONS

The identification and quantification of radiolytic products formed in the course of γ-irradiation of acetylsalicylic acid have been reported. The direct analysis

by HPLC revealed the presence of ppm levels of the two major radiolytic products, phenyl acetate (2) and *o*-hydroxyacetophenone (1). Their rate of formation increased with increasing irradiation dose in the range 5–15 Mrad.

The structure determination was performed by GC–MS. A specific extraction procedure was devised in order to concentrate other compounds as sub-ppm levels. The GC–MS analyses of the hexane extract showed the presence of twenty other trace compounds; the structures of six of them were confirmed unambiguously, and those of nine compounds were proposed on the basis of a single spectrum.

Decarboxylation leading to the formation of phenyl acetate (2) has been previously described as a mechanism in the radiation chemistry of salicylic acid in solution[1,3–4,6]. However, the formation of *o*-hydroxyacetophenone (1) resulting from the photo-Fries rearrangement (Scheme 1) has not previously been reported. The fact the photo-Fries rearrangement can be induced by γ-rays has been demonstrated in studies conducted on other substances[7].

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REFERENCES

1 A. M. Downes, *Aust. J. Chem.*, 11 (1958) 154–167.
2 I. Loeff and A. J. Swallow, *J. Phys. Chem.*, 68 (1964) 2470–2475.
3 C. B. Amphlett, G. E. Adams and B. D. Michael, *Adv. Chem. Ser.*, 81 (1968) 231–250.
4 R. W. Matthews and D. F. Sangster, *J. Phys. Chem.*, 69 (1965) 1938–1946.
5 L. R. de la Paz, *Philipp. Nucl. J.*, 4 (1976) 292–304.
6 S. G. Jivani and V. J. Stella, *J. Pharm. Sci.*, 74 (1985) 1274–1282.
7 D. Bellus, K. Schaffner and J. Hoigné, *Helv. Chim. Acta*, 51 (1968) 1980–1989.