

## Note

### Gas chromatographic identification of some chlorinated aromatic acids, chlorophenols, and their aromatic acid precursors

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Current reports suggest that stable volatile<sup>1,2</sup> and non-volatile<sup>3</sup> organochlorine compounds are nearly ubiquitous in chlorine-treated drinking water and river waters receiving chlorinated effluents. Although many organochlorine compounds originate from industrial and agricultural sources, it is likely that normal constituents of soils and natural waters, such as phenolic acids<sup>4</sup>, make up a large fraction of the dissolved organic matter reactive toward chlorine. Most of the non-volatile chlorine compounds identified in municipal sewage effluent in a recent study<sup>3</sup> (chlorinated pyrimidines, chlorinated aromatic acids) appear to be derived from natural precursors, and evidence suggests that chloroform and several other haloforms are formed by chlorination of naturally occurring humic materials<sup>5</sup>. The products of chlorination of natural substances may have important ecosystem effects; 5-chlorouracil (at 1 µg/l) significantly decreased the hatchability of carp eggs<sup>6</sup>.

We report a gas chromatographic (GC) method for separation and identification of fourteen chlorinated aromatic acids and their precursors, devised in the course of a study of their biological effects, and an application of the method in an investigation of the aqueous chlorination of mixtures of aromatic acids.

#### EXPERIMENTAL

The acids used were obtained from J. T. Baker (Phillipsburgh, N.J., U.S.A.), Sigma (St. Louis, Mo., U.S.A.), Aldrich (Milwaukee, Wisc., U.S.A.), and Pfaltz & Bauer (Stamford, Conn., U.S.A.) and were of the highest purity available. 3,5-Dichlorosalicylic acid was recrystallized (water) before use. 5-Chlorovanillic acid was synthesized from 5-chlorovanillin using a published method<sup>7</sup>; the product, after recrystallization from water, had a m.p. of 248° (ref. 7: 244-245°).

Hypochlorite solution used for chlorination was commercial Clorox® bleaching solution, nominally 5.25% NaOCl (verified iodometrically).

Trimethylsilylated derivatives of the aromatic acids were synthesized by weighing 1-2 mg of the pure acid and a similar amount of benzoic acid (internal standard) into a dry sampling vial with a PTFE-faced seal and adding 1.0 ml of Tri-Sil® (Pierce, Rockford, Ill., U.S.A.). After thorough vortex mixing (*ca.* 30 sec, ambient

temperature) the sample was ready for injection. None of the acids tested required heating or extended reaction times for derivative formation.

The gas chromatograph used was a Hewlett-Packard 7620 equipped with dual-flame detectors, automatic injector, electronic integrator, and 25-cm recorder. Glass and stainless-steel (1.83 m  $\times$  6.3 mm I.D.) columns, containing 3% SP2401 silicone on acid-washed 80–120 mesh Supelcoport (Supelco, Bellefonte, Pa., U.S.A.) were used. The retention characteristics of the columns were very similar.

Gas flow-rates to the flame detector were 300 ml/min of air and 40 ml/min of hydrogen. Carrier gas (nitrogen) flow-rate was 60 ml/min. The injection port was held at 200° and the detector at 280°. The automatic sampler injected 4.5  $\mu$ l of solution for each run. A temperature program of 100°–200° at 6°/min was employed.

In a series of competition experiments, excess molar quantities of unchlorinated aromatic acids were treated with NaOCl at a concentration similar to that employed in waste treatment systems. In a typical experiment, salicylic, vanillic, and phenylacetic acids (10 mg each) and 100 mg of *p*-hydroxybenzoic acid were dissolved in 500 ml of water. Aqueous hypochlorite was added to establish a concentration of 30 mg NaOCl per l. The solution was stirred for 60 min, and excess NaOCl (if present) was destroyed with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The solution was acidified to pH 3 (10% HCl), extracted (2  $\times$  50 ml EtOAc), dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated almost to dryness under reduced pressure, transferred to a GC vial and trimethylsilylated in the presence of benzoic acid.

## RESULTS AND DISCUSSION

Fourteen different aromatic acids (including benzoic acid as an internal standard) were individually converted to their trimethylsilyl (TMS) esters. The acids chosen were those most commonly reported in soils, fresh and salt waters, and waste effluents. Multiple injections of each acid established its retention time relative to benzoic acid (Table I). Only the 3-chloro-4-hydroxybenzoic acid and vanillic acid

TABLE I

### RELATIVE RETENTION TIMES (RRT) OF TRIMETHYLSILYL DERIVATIVES OF FOURTEEN AROMATIC ACIDS

RRTs are means of 13 determinations.

No.	Acid	RRT $\pm$ standard deviation
1	Benzoic	1.00
2	Phenylacetic	1.19 $\pm$ 0.00
3	Salicylic	1.87 $\pm$ 0.00
4	4-Chlorophenylacetic	1.94 $\pm$ 0.00
5	<i>o</i> -Hydroxyphenylacetic	2.16 $\pm$ 0.02
6	<i>m</i> -Hydroxyphenylacetic	2.34 $\pm$ 0.01
7	<i>p</i> -Hydroxybenzoic	2.42 $\pm$ 0.03
8	5-Chlorosalicylic	2.55 $\pm$ 0.01
9	3,5-Dichlorosalicylic	2.86 $\pm$ 0.02
10	Vanillic	2.93 $\pm$ 0.02
11	3-Chloro-4-hydroxybenzoic	2.95 $\pm$ 0.03
12	3,5-Dichloro-4-hydroxybenzoic	3.30 $\pm$ 0.03
13	Syringic	3.44 $\pm$ 0.02
14	5-Chlorovanillic	3.50 $\pm$ 0.01

esters were not completely resolved. Distinction between these compounds would probably be possible using electron capture GC, but this was not investigated.

An equimolar mixture of the fourteen acids was prepared, derivatized, and injected; Fig. 1 shows the resulting GC trace.

Reaction of a mixture of *p*-hydroxybenzoic, salicylic, vanillic and phenylacetic acids with less than one equivalent of NaOCl gave the GC trace shown in Fig. 2. Production of 5-chlorovanillic acid, 3,5-dichloro-4-hydroxybenzoic acid, and (probably) 3-chloro-4-hydroxybenzoic acid was observed. A relatively larger amount of 5-chlorosalicylic acid was formed, but no dichloro derivative could be detected. Benzoic and phenylacetic acids did not afford measurable amounts of chlorination products. The results are consistent with a requirement for activation of the aromatic nucleus by electron-donating substituents (HO-, CH<sub>3</sub>O-) for aqueous chlorination to occur<sup>8</sup>.

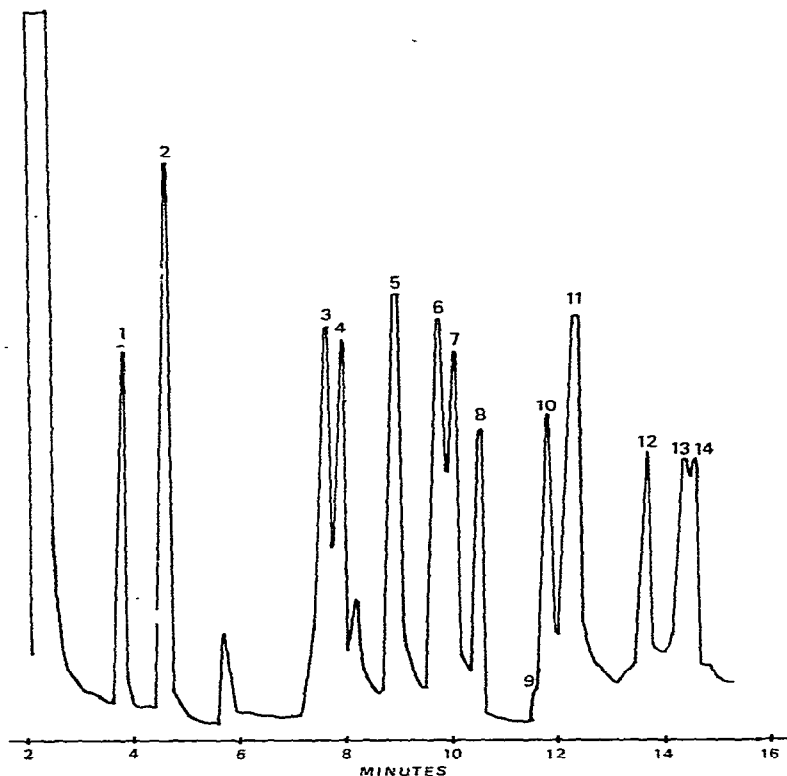


Fig. 1. Separation of chlorinated aromatic acids and their precursors. Numbers refer to acids in Table I. GC conditions, see text. 10 nmoles of each acid were injected.

In addition to peaks due to the expected compounds, six additional peaks were observed in Fig. 2. Chlorination of each individual acid of the mixture, and comparison of the retention times of the product peaks with those of various simple TMS phenols, suggested that three of the newly observed peaks were due to 4-chloro-

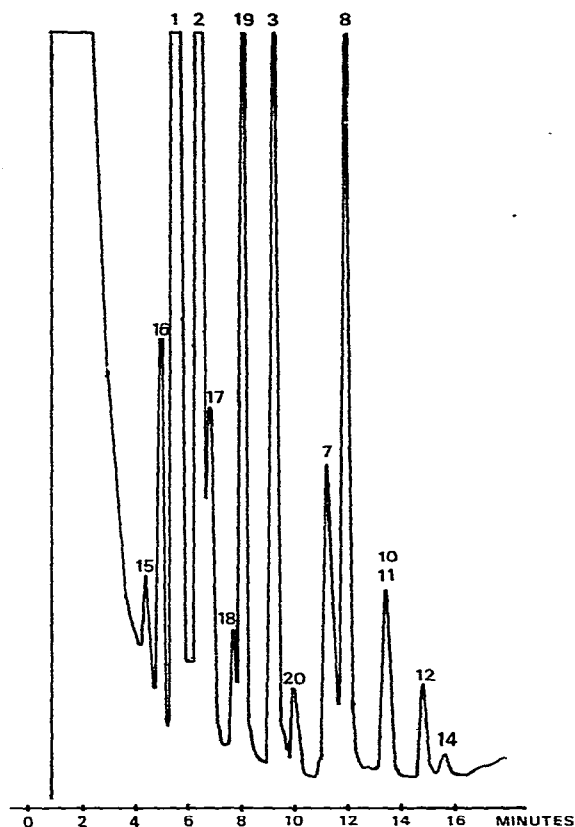


Fig. 2. Chlorination of a mixture of aromatic acids. Conditions, see text. Numbers up to 14 refer to acids in Table I. Tentative identification of additional peaks: 15 = 2-chlorophenol; 16 = 4-chlorophenol; 17 = 2,4-dichlorophenol; 18 = unknown (derived from vanillic acid); 19 = 2,4,6-trichlorophenol; 20 = unknown (derived from vanillic acid).

phenol (derived from *p*-hydroxybenzoic acid by an apparent hypochlorite-induced decarboxylation), 2,4-dichlorophenol, and 2,4,6-trichlorophenol. Two other peaks (possibly also due to chlorinated phenols) were derived from vanillic acid. The origin of the sixth peak, which has the same retention time as the TMS derivative of 2-chlorophenol, is uncertain; it was not produced when any of the acids (including salicylic or *p*-hydroxybenzoic) were chlorinated individually. The results imply that some of the chlorophenols in water supplies may be derived in part from naturally occurring phenolic acids.

#### ACKNOWLEDGEMENT

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