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CAPILLARY GAS CHROMATOGRAPHY OF DIHYDROXYBENZOIC, -PHENYLACETIC AND -PHENYLPROPIONIC ACIDS

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SUMMARY

All six possible ring-substituted dimethoxy and dihydroxy isomers of phenylacetic and β-phenylpropionic acids were prepared. The gas chromatographic (GC) separation, on an SE-54 coated fused-silica capillary column, of all 24 isomers (as their trimethylsilyl derivatives) is presented for the first time, as well as the first reported synthesis of 2,6-dihydroxyphenylacetic acid. The GC elution sequence of silylated dihydroxyphenylacetic and dihydroxyphenylpropionic acids is compared to that of dihydroxybenzoic acids. Mass spectral (MS) fragmentation patterns of the silylated isomers reflect positions of the substituents and can be used to characterize isomers. The data presented will allow the determination of these isomeric acids, based on their MS and GC data, relative to common, commercially available isomers. The developed data were applied to identifications of unknown phenolic acids of tobacco smoke.

INTRODUCTION

Due to their almost universal occurrence in plants, there exists a large body of research on phenolic acids[1]. Recently, increased interest in phenolic acids has been focused on their plant growth-regulating properties, such as seed germination inhibition[2–7]. Phenolic acids are also important in animal systems, because of their relationship to the biologically active catecholamines[8] and increased levels of various isomers are found in the urine of patients with certain metabolic diseases[9–12]. Traditionally, phenolic acids have been analyzed by paper[13,14] or thin-layer[1] chromatography. Recently, however, gas chromatography (GC)[10,12,15–17] and high-performance liquid chromatography[11,18] have been used more effectively.

Our interest in phenolic acids stems from their structural relationship to a major cigarette smoke co-carcinogen, catechol, and from the possible contribution of phenolic acids to the tumorigenicity of cigarette smoke. Recently, we developed a new method for the isolation of phenolic acids by lipophilic gel adsorption chromatography on Sephadex LH-20 with methanol–chloroform solvents, and have successfully applied it to phenolics of tobacco[19] and tobacco smoke[20]. Capillary GC–

mass spectrometry (MS) indicated the presence of several isomeric dihydroxyphenylacetic and -phenylpropionic acids in the tobacco smoke isolate. Since standards were not available for all the isomeric dihydroxy acids, we undertook to prepare all the possible isomers of dihydroxyphenylacetic and -phenylpropionic acids* (via the corresponding dimethoxy acids) and to determine, for the first time, the capillary GC elution order of their trimethylsilyl derivatives. The synthesis of 2,6-DHPA is reported for the first time.

EXPERIMENTAL**

Solvents were Burdick & Jackson (Muskegon, MI, U.S.A.) "distilled-in-glass" grade. 2,3-, 2,4- and 3,5-Dimethoxybenzaldehyde; 2,5- and 3,4-dimethoxyphenylacetic acid; 2,5- and 3,5-dimethoxycinnamic acid; 2,6-dimethoxybenzonitrile; 2,5-DHPA, all six possible isomers of DHBZ, pyridine hydrochloride and hippuric acid were obtained from Aldrich (Milwaukee, WI, U.S.A.). 2,3- and 2,4-Dimethoxycinnamic acids were obtained from K & K Labs. (Plainview, NY, U.S.A.). 3,4-DHPP was obtained from Fluka (Hauppauge, NY, U.S.A.). Hydrogen peroxide (30%) was obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). All chemicals and reagents were used without further purification.

Equipment

The ^{1}H NMR spectra were obtained using a JEOL PS/PFT 100 NMR spectrometer in the Fourier transform mode. The spectra were obtained at 100 MHz using a deuterium lock set at 15.4 MHz and referenced to tetramethylsilane. Samples were dissolved in hexadeuteroacetone at the 4% level in standard 5-mm tubes. Spectra were taken using a 5.0 sec pulse repetition rate with a 90° pulse angle (20 μsec) and a 1.5 kHz spectral window; 64 spectral scans were accumulated in 8K of computer core.

MS data for the trimethylsilyl derivatives were obtained with a Hewlett-Packard 5985B GC–MS system. Silylated acids were introduced into the mass spectrometer after GC separation on a 25 m × 0.3 mm I.D. SE-54 fused-silica capillary column. The gas chromatograph oven temperature was programmed from 80–280°C at 2°C/min; 35 cm/sec helium flow (scan rate 400 a.m.u./sec; scan range 50–500 a.m.u.). Probe mass spectra of neat standards were obtained with the spectrometer operated in the direct-insertion probe mode (scan rate 266.7 a.m.u./sec; probe temperature 20°C for 2 min, then 20°C to 200°C at 20°C/min; ion source temperature 200°C; electron multiplier 1600 V; scan range 40–300 a.m.u.

Synthesis

2,6-Dimethoxybenzaldehyde. An attempt to reduce 2,6-dimethoxybenzonitrile to the aldehyde with lithium triethoxyaluminohydride according to the procedure of

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* It will be understood that dihydroxyphenylpropionic acid refers to β-(dihydroxyphenyl)propionic acid. The following abbreviations will be used in the text: DHBZ = dihydroxybenzoic acid; DHPA = dihydroxyphenylacetic acid and DHPP = β-(dihydroxyphenyl)propionic acid. Positions of hydroxyls on the aromatic ring will be indicated by numbers.

** Reference to a company or product name does not imply approval or recommendation by United States Department of Agriculture.

Brown and Garg[21] resulted in only starting material being recovered. 2,6-Dimethoxybenzaldehyde was successfully prepared by Raney nickel reduction of 2,6-dimethoxybenzonitrile according to the procedure of Staskun and Backeberg[22], except that 88% formic acid was used and the reduction was repeated. From 20 g of nitrile, 11 g (54%) of crude product was isolated; recrystallization from diethyl ether–light petroleum (b.p. 30–60°C), m.p. 93–94°C (lit.[23], 96–97°C); MS (*m/z*, %): 166, 100 [M^+]; 165, 47 [$M^+ - 1$]; 106, 63 [$M^+ - OCH_3 - HCO$]; 75, 54.

Dimethoxyphenylacetic acids. 2,3-, 2,4-, 2,6- and 3,5-Dimethoxyphenylacetic acids were prepared via the azalactones and phenylpyruvic acids[24]. The corresponding aldehyde was condensed with hippuric acid, acetic anhydride and anhydrous sodium acetate to give the azalactone, which was hydrolyzed with aqueous sodium hydroxide. The resulting mixture of dimethoxyphenylpyruvic and benzoic acids was separated by saturating the solution with sulfur dioxide and filtering the precipitated benzoic acid. After acidification, the separated phenylpyruvic acid was oxidized with cold, basic 30% hydrogen peroxide to the corresponding dimethoxyphenylacetic acid. A convenient method for recrystallizing the dimethoxyphenylacetic acids was found to consist of repeated extraction of the crude acids with fresh boiling isooctane. On cooling in a refrigerator, the combined extracts gave the pure acids. A second crop of crystalline product could be obtained by boiling the filtrate to reduce the solution volume, until crystals began to form; the solution was then allowed to cool. Melting points of azalactone, phenylpyruvic acid, and phenylacetic acid were, respectively: 2,3-dimethoxy- 165–166°C (benzene) (lit.[25], 170°C), 146–148°C (benzene) (lit.[25], 145°C), 80–81°C (light petroleum) (lit.[26], 81–82°C; lit.[27], 81–81.5°C); 2,4-dimethoxy- 165–168°C (lit.[28], 182°C), 152–154°C (benzene) or 156–160°C (glacial acetic acid), 107–108°C (lit.[29], 107°C; lit.[30], 107–107.5°C); 2,6-dimethoxy- 117–117.5°C (ethanol), -(no m.p.), 151–154°C (benzene) (lit.[26], 149.5–152.5°C; lit.[27], 154°C); 3,5-dimethoxy- 149–150°C, 178–180°C, 101–102°C (lit.[26], 100–102°C, lit.[27], 101°C).

2,6-Dimethoxyphenylacetic acid (alternative method). 2,6-Dimethoxyphenylacetic acid was prepared by oxidizing a mixture of *m*-dimethoxybenzene in acetic anhydride with potassium permanganate according to the procedure of Buess[31]. The crude product (m.p. 118–125°C), contrary to the literature[31], contained both 2,4- (56%) and 2,6-dimethoxyphenylacetic (39%) acids, as determined by GC. After treatment with base and steam distillation to remove unreacted *m*-dimethoxybenzene, the acidified solution gave a precipitate with a composition of 23% 2,4- and 76% 2,6-dimethoxyphenylacetic acids. The mixed isomers (3.5 g in 40 ml benzene) were allowed to stand for several days at 8°C and 1.56 g (m.p. 152–154°C) of pure 2,6-dimethoxyphenylacetic acid was obtained.

2,6-Dimethoxycinnamic acid. 2,6-Dimethoxycinnamic acid was prepared by the Perkin reaction according to the procedure reported by Johnson for β-piperonylacrylic acid[32]. 2,6-Dimethoxybenzaldehyde (3.5 g, 0.021 mol), malonic acid (4.21 g, 0.0405 mol), pyridine (8.4 ml, 0.1 mol) and piperidine (0.21 ml, 0.0021 mol) were refluxed for 1.5 h. The resulting mixture was cooled and then poured into a mixture of 15 ml concentrated hydrochloric acid and 30 g of chopped ice. The precipitate was filtered and washed with 10 ml of 10% hydrochloric acid and 2 × 10 ml water (yield: 3 g, 68%). Attempts to recrystallize the crude acid with diethyl ether–light petroleum or ethanol failed to yield purified material. The crude 2,6-dimethoxycinnamic acid was purified by silicic acid column chromatography. The acid (3.0 g) was placed on

the top of a 100 g silicic acid column (Mallinckrodt, 100 mesh silicic acid, washed with methanol to remove fines and activated at 155°C for 16 h) packed in light petroleum. The column was eluted with 1 l of benzene followed by 1 l of diethyl ether–benzene (20:80), which eluted the compound of interest. After evaporation of the solvent, the purified 2,6-dimethoxycinnamic acid was recrystallized from diethyl ether (yield 2.49 g, 56.6%, m.p. 141–143°C; lit.[33], 146–147°C; lit.[34], 151–153°C; MS (*m/z*, %): 208, 72 [M^+]; 191, 4 [$M^+ - OH$]; 177, 100 [$M^+ - OCH_3$]; 176, 10 [191 $- CH_3$]; 162, 11 [177 $- CH_3$]; 149, 34 [177 $-$ CO]; 148, 26 [176 $-$ CO]; 133, 26 [177 $- CO_2$]).

β-(Dimethoxyphenyl)propionic acids. 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-dimethoxyphenylpropionic acids were prepared by hydrogenation of the respective cinnamic acids in 95% ethanol, with 10% palladium on charcoal, at room temperature and atmospheric pressure. Ratio of materials was: acid–ethanol–catalyst (1:50:0.5, w/v/w). Nitrogen was passed through the solution to expel air and then hydrogen was bubbled for 3 h, with magnetic stirring. The catalyst was filtered off and the solvent evaporated to give crude yields of 85–90%. Melting points of the dimethoxyphenylpropionic acids were respectively: 2,3-, 64–65°C (lit.[35], 68°C); 2,4-, 100–101°C (lit.[36], 103–104°C); 2,5-, 63–64°C (lit.[37], 65–66°C); 2,6-, (from hexane) 107–108°C (lit.[38], 109°C); 3,5-, 60–61°C (lit.[39], 61–62°C).

DHPA and DHPP. 2,3-, 2,4- and 3,5-DHPA and 2,3-, 2,4-, 2,5-, and 3,5-DHPP were prepared by demethylation of the corresponding dimethoxy acids, using pyridine hydrochloride, according to the procedure of Blakely and Simpson[40]. Melting points of recrystallized DHPA were respectively: 2,3-, 88–90°C (from benzene or hexane) (lit.[41], 103–104°C), mass spectra identical to literature[42] (heating the acid converted it to 2,3-DHPA lactone, recrystallized from water, m.p. 187–188°C; lit.[43], 189°C); 2,4- 116–118°C (from benzene–diethyl ether) (lit.[44], 112–114°C); 3,5-, 123–124°C (from benzene) (lit.[45], 128–128.5°C). Melting points of the recrystallized DHPP were respectively (from diethyl ether–light petroleum unless otherwise specified): 2,3-, 124–125°C (lit.[40], 125–127°C); 2,4-, 163–164°C (lit.[46], 165°C); 2,5-, 128.5–129°C (lit.[47], 129–131°C); 3,5-, 143–144°C (from ethyl acetate) (lit.[39], 142–143°C).

2,6-DHPA and lactone. 2,6-Dimethoxyphenylacetic acid (1 g), in a 25-ml round bottom flask, was heated with pyridine hydrochloride (5 g) for 30 min, with an air condensor and drying tube attached, according to the procedure of Blakely and Simpson[40]. After allowing the reaction mixture to cool, 30 ml of a saturated aqueous sodium hydrogen carbonate solution were poured down the condensor and the solid residue broken up with a spatula. Contrary to the demethylations described before, the residue did not completely dissolve in the sodium hydrogen carbonate solution. The aqueous sodium hydrogen carbonate was transferred to a 60-ml separatory funnel and the remaining solid residue was dissolved in diethyl ether (about 50 ml) and transferred to a 125-ml separatory funnel. The sodium hydrogen carbonate and diethyl ether solutions were each cross-extracted with 2 × 20 ml of saturated aqueous sodium hydrogen carbonate and diethyl ether, respectively, with the extracts added to the proper original solutions. The aqueous sodium hydrogen carbonate solution was adjusted to pH 3 with conc. hydrochloric acid, extracted with diethyl ether and the ether evaporated to give 0.59 g (69%) of an oil. Attempts to recrystallize the crude acid from diethyl ether–light petroleum failed due to ethanol preservative in the ether. Pure 2,6-DHPA was obtained by adding 30 ml benzene to the crude acid

(0.59 g) plus sufficient diethyl ether to dissolve the residue and then evaporating the mixture on a rotary evaporator just until crystals began to appear in the round-bottom flask. The flask was removed and allowed to sit overnight at 8°C from which 0.35 g (41%) of pure 2,6-DHPA was obtained: light tan crystals m.p. 157–158°C; MS (*m/z*, %): 168, 42 [M^+]; 150, 30 [$M^+ - H_2O$]; 123, 29 [M^+ – COOH]; 122, 100 [150 – CO]; 94, 40 [122 – CO]; 77, 11; 66, 22. NMR (ppm): 3.67s, 2H (CH_2); 6.39d (J = 8.1 Hz), 2H (*m*-ring H); 6.88t (J = 8.1 Hz), 1H (*p*-ring H); 8.19bs, 3H (COOH, 2-OH).

The diethyl ether solution from the demethylation reaction was extracted with 6 *M* hydrochloric acid (3 × 20 ml), followed by water (1 × 20 ml). The diethyl ether was dried over anhydrous sodium sulfate, filtered and evaporated to give 0.34 g of crude lactone. The lactone was recrystallized from a benzene–diethyl ether solution as described for the acid and yielded 0.12 g of pure 2,6-DHPA lactone (4-hydroxy-2,3-dihydrobenzo[*b*]furan-2-one) m.p. 178–179°C (lit.[43], 179–180.5°C); MS (*m/z*, %): 150, 100 [M^+]; 122, 99 [M^+ – CO]; 94, 74 [122 – CO]; 66, 54. The lactone (0.11 g) was refluxed with 30 ml of water for 30 min, and the water evaporated on a rotary evaporator. Recrystallization of the residue from benzene–diethyl ether as above yielded 0.04 g of 2,6-DHPA, m.p. 157–158°C.

2,6-DHPP and lactone. 2,6-DHPP (1 g) was demethylated as described above for 2,6-DHPA except that only a very small amount of solid material would not dissolve in the aqueous sodium hydrogen carbonate solution, indicative of only a minor amount of lactone formed. The crude acid (0.63 g of light yellow oil) was recrystallized from benzene–diethyl ether as described above for the corresponding phenylacetic acid. The pure 2,6-DHPP (0.36 g, 41% yield) had a m.p. of 115–117°C (with decomposition by gas evolution) (lit.[48], 174–176°C); MS (*m/z*, %): 182, 59 [M^+]; 164, 77 [$M^+ - H_2O$]; 136, 78 [164 – CO]; 135, 21; 123, 32 [M^+ – CH_2COOH]; 122, 100 [164 – CH_2CO]; 107, 16; 94, 34; NMR (ppm): 2.61t and 2.93t (J = 6.6 Hz), 4H (–CH_2CH_2–); 6.39d (J = 7.8 Hz), 2H (*m*-ring H); 6.85t (J = 7.8 Hz), 1H (*p*-ring H); 8.47bs, 3H (COOH, 2-OH). The melted acid resolidified after several minutes at 120°C to form the lactone and remelted with sublimation at 167–168°C (lit.[48], 224–225°C), MS (*m/z*, %): 164, 100 [M^+]; 136, 44 [M^+ – CO]; 122, 78 [M^+ – CH_2CO]; 107, 15; 94, 37.

Capillary GC. Samples of the acids were converted to their trimethylsilyl derivatives by heating them at 75°C for 20 min with an equal volume mixture of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N′,N′-dimethylformamide (DMF). The glass capillary column was a 25 m × 0.3 mm I.D. fused-silica column coated with SE-54. The column was prepared by the method of Arrendale *et al.*[49] and consisted of a Superox-4 pretreatment and deactivation step followed by an intermediate layer of Emulphor ON-870 and then coated with a solution of 3 mg/ml of SE-54 in dichloromethane. All coatings were done by the static method. The instrument was a Hewlett-Packard 5830 gas chromatograph modified with an all-glass split injector system[50]. The column was operated with hydrogen carrier gas (column head pressure 5 p.s.i., giving 35 cm/sec linear velocity), temperature program 80–250°C at 2°C/min; injector 250°C; detector 310°C.

RESULTS AND DISCUSSION

All isomers of DHBZ, DHPA and DHPP were either obtained commercially

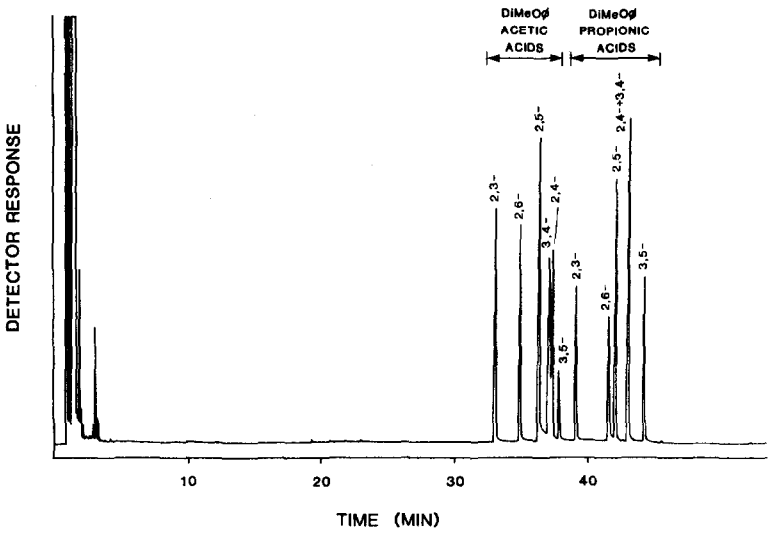

Fig. 1. Gas chromatogram of TMS derivatives of dimethoxyphenyl (DiMeOØ) acetic and -propionic acids.

or synthesized. Discrepancies were found between the melting points of synthesized 2,3-DHPA and 2,6-DHPP and the literature; however, MS and NMR data, along with conversion of the synthesized compounds to the corresponding lactones, confirmed the assigned structures. The synthesis of 2,6-DHPA is being reported for the first time. Yoshizako and co-workers[51–53] reported that 2,6-DHPA is a metabolite of *Aspergillus fumigatus*, however, they identified the compound as the dimethoxy-methyl ester.

The isomeric dimethoxy and dihydroxy acids were converted to their trimethylsilyl (TMS) derivatives in preparation for capillary GC on a fused-silica column coated with SE-54. The silylation reagent consisted of equal portions of BSTFA and DMF. It was found that heating the samples with reagent to 75°C for 20 min converted all the hydroxyl groups to their TMS derivatives and gave only one GC peak per isomer.

Since all of the isomers of dimethoxyphenylacetic and -propionic acid were on hand, it was of interest to compare their GC elution order in relation to the dihydroxy isomers. The GC elution order of all 12 dimethoxy isomers is shown in Fig. 1. The order of elution of the isomers was the same in both series, except that the 2,4- and 3,4-dimethoxyphenylpropionic acids coeluted.

The GC elution of all 12 DHPA and DHPP, as TMS derivatives, is shown in Fig. 2. The order of elution of the isomers was the same in both series but different from the dimethoxy analogues in that reversals in elution occurred between the 2,3- and 2,6-isomers and the 2,5- and 3,4-isomers. All isomers separated well except for the 2,4- and 2,5-DHPA. A somewhat better separation between the 2,4- and 2,5-DHPA was achieved by a slower temperature program, as shown in Fig. 3.

The capillary GC separation of the TMS derivatives of all 18 possible isomers of DHBZ, DHPA and DHPP is given in Fig. 4. The benzoic acids partially overlapped the phenylacetic acids. The 2,3- and 2,6-DHBZ and the 2,4- and 3,4-DHBZ

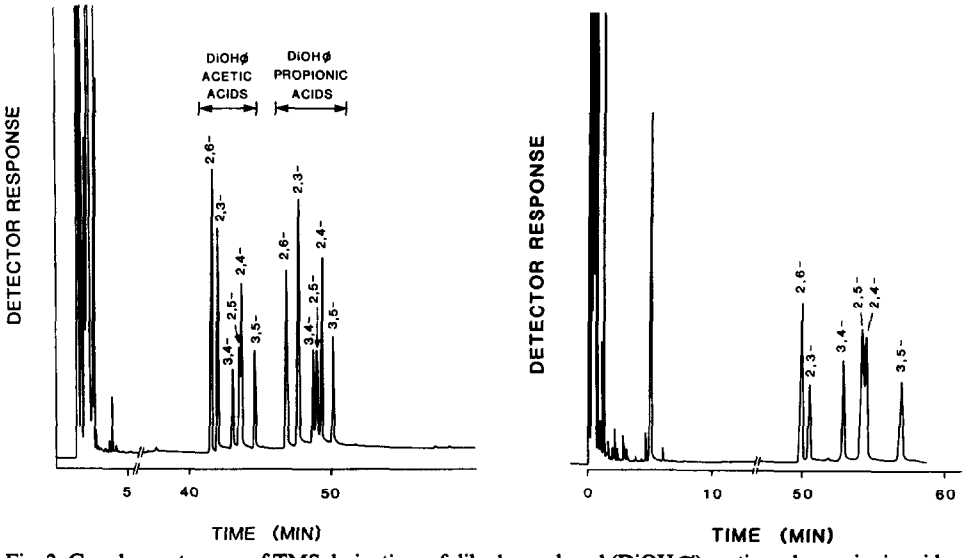

Fig. 2. Gas chromatogram of TMS derivatives of dihydroxyphenyl (DiOHØ) acetic and -propionic acids.

Fig. 3. Gas chromatogram of TMS derivatives of DHPA. Column: 25 m × 0.3 mm I.D. fused-silica SE-54. Temperature programmed from 100 to 150°C at 1°C/min.

isomers elution order was reserved compared to that of the phenylacetic and phenylpropionic acids. The elution order of the 2,3-, 2,5-, and 2,6-DHBZ on the SE-54 capillary column was the same as that found by Morita[15] with an OV-1 packed column. However, whereas packed columns (OV-1[15] and OV-17[54]) failed to separate

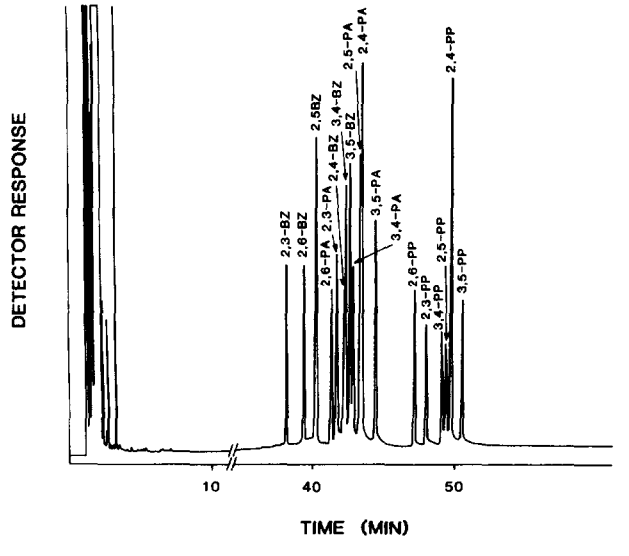

Fig. 4. Gas chromatogram of TMS derivatives of all 18 isomers of DHBZ, DHPA and DHPP. BZ = Benzoic; PA = phenylacetic; PP = phenylpropionic.

TABLE I

GC ELUTION ORDER AND CHARACTERISTIC MS IONS FOR SILYLATED DIHYDROXY AROMATIC ACIDS

RRT = Relative retention times relative to 3,4-DHPA. Ions selected for identifying characteristics: ** base peak; * useful intense ions; percent of base peak in parentheses, normalized on most intense ion above 100 a.m.u.

Name	*Commercially available*	*RRT*	*Group*	*Selected identifying MS ions of TMS derivatives*
2,3-DHBZ	+	0.891		355**,193(40)
2,6-DHBZ	+	0.919		355**,267(29)
2,5-DHBZ	+	0.941		355**
2,6-DHPA		0.965	I	384(42),341*(85),267(66),237(62),147**
2,3-DHPA		0.974	I	384(23),369(23),253(26),179*(96),147**
2,4-DHBZ	+	0.986		355**,281(12)
3,4-DHBZ	+	0.988		370(41),355(24),193**
3,5-DHBZ	+	0.995		370**,355*(96)
3,4-DHPA	+	1.000		384*(77),267*(85),179**
2,5-DHPA	+	1.012		384**,341(66),147*(83)
2,4-DHPA		1.016	II	384(13),341(10),267**
3,5-DHPA		1.037	II	384(44),369(22),252(40),147**
2,6-DHPP		1.101	III	398(35),383(42),341(67),280(48),267**,147(76)
2,3-DHPP		1.120	III	398(18),383(18),193(14),179**,147(37)
p-Coumaric	+	1.133		308(64),293*(95),249(54),219**
3,4-DHPP	+	1.146		398(29),267(33),179**
2,5-DHPP		1.153	IV	398**,280(46),147(99)
2,4-DHPP		1.162	IV	398(16),267**
3,5-DHPP		1.180	IV	398(35),294(38),281**

the remaining isomers (2,4-, 3,4-, 3,5-), the SE-54 capillary column resolved the 3,5-isomer and partially resolved the 2,4- and 3,4-DHBZ.

All of the isomers of dihydroxybenzoic acid are commercially available, as well as 2,5- and 3,4-DHPA, and 3,4-DHPP. The available *p*-hydroxycinnamic (*p*-coumaric) acid elutes between 2,3- and 3,4-DHPP. The relative GC retention times of these nine acids, together with the tabulated GC–MS data (Table I) can be used to determine the isomer composition of unknown acids, without the need to synthesize all of the isomers. Of the four commercially unavailable DHPA isomers, GC elution in relation to available acids will narrow the possible choices to 2,6- and 2,3-DHPA in one group (group I, Table I) eluting between 2,5- and 2,4-DHBZ, and 2,4- and 3,5-DHPA in another group (group II, Table I) eluting after 2,5-DHPA. MS analyses will readily distinguish which isomers are present in group I since the 2,6-DHPA isomer has a prominent TMS fragment ion at *m/z* 341, which is absent in the 2,3-isomer. Similarly, the isomers in group II can be distinguished by the intensities of their molecular ions and the fact that 2,4-DHPA exhibits a base peak of *m/z* 267, *versus* 147 for 3,5-DHPA.

Although only 3,4-DHPP is commercially available, when combined with *p*-coumaric acid, they will define two GC elution groups for the remaining DHPP isomers. As with the phenylacetic acids, the first group (group III, Table I), eluting before *p*-coumaric acid, contains 2,6- and 2,3-DHPP, which can easily be differen-

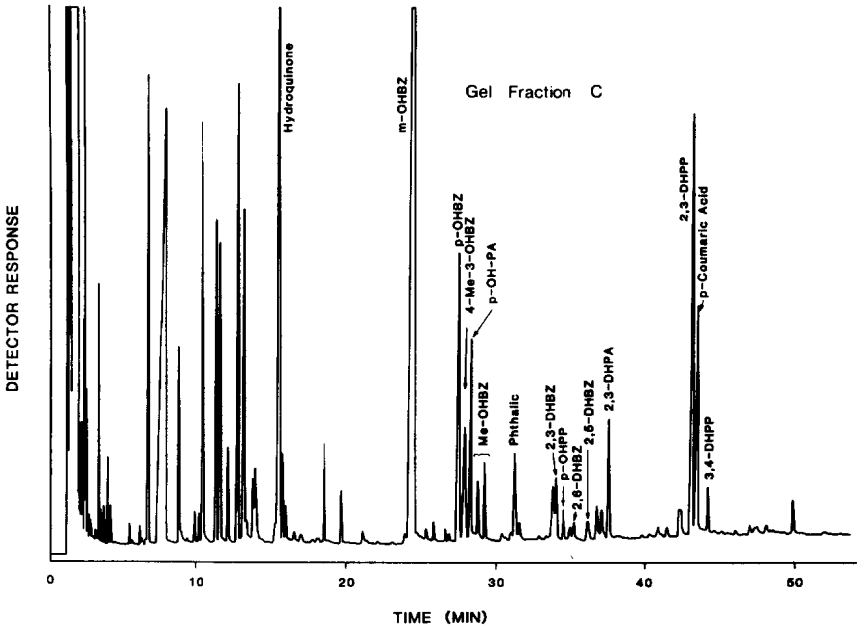

Fig. 5. Gas chromatogram of a gel chromatography fraction (Fraction C) of cigarette smoke phenolics, as TMS derivatives[20]. Me = Methyl; see Fig. 4 for other abbreviations.

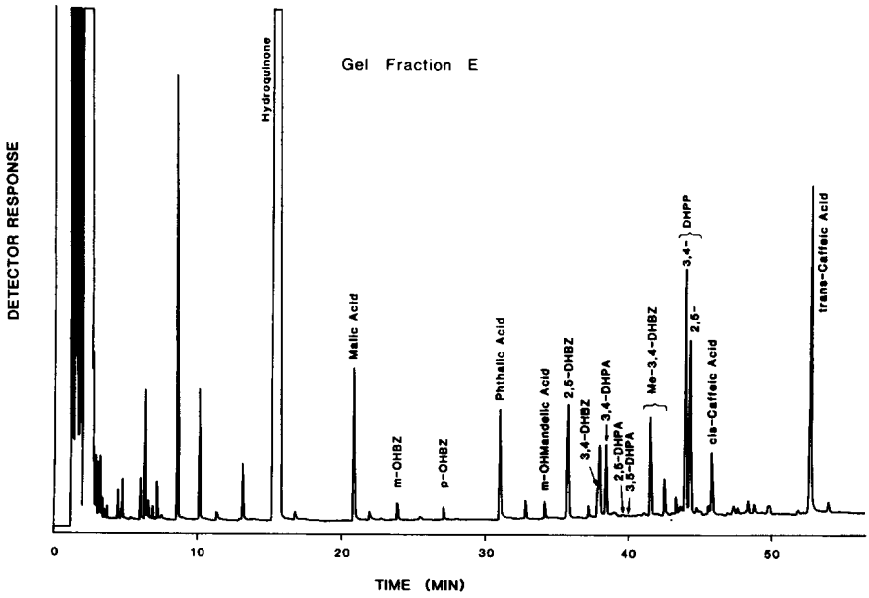

Fig. 6. Gas chromatogram of a gel chromatography fraction (Fraction E) of cigarette smoke phenolics, as TMS derivatives[20]. See Fig. 4 for abbreviations.

tiated by their different MS base peaks (Table I). The other group of DHPP isomers (group IV, Table I), eluting after the 3,4-DHPP, contains the 2,5-, 2,4- and 3,5-isomers. Again, each isomer exhibits different MS base peaks (Table I), which will characterize that particular isomer.

We have applied the above combination of GC relative retention times and MS data to the identification of unknown phenolic acids to an acid fraction of cigarette smoke condensate[20]. GC separation of Sephadex LH-20-purified cigarette smoke phenolic fractions (Figs. 5 and 6) led to the identification of 2,3-, 2,5-, 3,4- and 3,5-DHPA acids and 2,3-, 2,5- and 3,4-DHPP.

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