The results confirm the structure of η-pyrromycinone (Ⅲ) and also the carbon skeletons of ε-pyrromycinone (Ⅳ),[4] ζ-pyrromycinone (Ⅴ),[4] ε-isorhodomycinone (Ⅵ)[5] and ζ-isorhodomycinone (Ⅶ),[5] since these anthracyclinones have been related to Ⅲ.

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Faculty of Pharmaceutical Sciences,
Osaka University,
Toneyama, Toyonaka, Osaka

Zen-ichi Horii (堀井善一)
Takefumi Momose (百瀬雄章)
Yasumitsu Tamura (田村恭光)

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4) H. Brockmann, V. Prelog, W. D. Ollis, *et al.*: Tetrahedron Letters, No. 8, 25 (1960).
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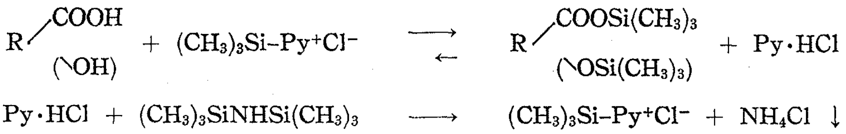
Gas Chromatography of Aromatic Acids

Gas chromatographic separation of aromatic acids usually requires conversion of the acids to suitable volatile derivatives. This has been carried out by treating the acids with diazomethane, and thus, a number of methyl esters of aromatic acids have been chromatographed satisfactorily.[1,2] However, the application of this method to determination of acids with two phenolic hydroxyl groups such as protocatechuic acid was unsuccessful because of the conversion by diazomethane to a mixture of derivatives.[2] In order to solve this problem, an improved method[3] which involves acetylation of phenolic groups, followed by methylation, has been devised, but the method is tedious and time consuming.

We have now developed a rapid and convenient method which is widely applicable to separation and determination of a variety of aromatic acids including phenolic acids. This development has stemmed from our recent finding[4] that carboxylic acids can be readily and quantitatively converted to their trimethylsilyl (TMS) esters by the use of the convenient silylation procedure.[5,6]

The aromatic acids were converted to their TMS derivatives in the following manner: 1~5 mg. of the acid was placed into a small stoppered vial and dissolved in 0.8 ml. of dry pyridine. To this solution was added 0.1 ml. of trimethylchlorosilane, followed by 0.1 ml. of hexamethyldisilazane. The reaction sequence may be written as follows:



$$R\langle{}^{COOH}_{(OH)} + (CH_3)_3Si\text{-}Py^+Cl^- \rightleftarrows R\langle{}^{COOSi(CH_3)_3}_{(OSi(CH_3)_3)} + Py\cdot HCl$$

$$Py\cdot HCl + (CH_3)_3SiNHSi(CH_3)_3 \longrightarrow (CH_3)_3Si\text{-}Py^+Cl^- + NH_4Cl \downarrow$$

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1) C. C. Sweeley, C. M. Williams: Anal. Biochem., 2, 83 (1961).
2) C. M. Williams: *Ibid.*, 4, 423 (1962).
3) C. M. Williams, R. H. Leonard: *Ibid.*, 5, 362 (1963).
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5) M. Makita, W. W. Wells: Anal. Biochem., 5, 523 (1963).
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The formation of the TMS derivatives occurred very rapidly at room temperature, and within a few minutes the entire reaction mixture could be injected directly into the chromatograph. The TMS derivatives thus prepared were generally stable and volatile enough for gas chromatography. Identification of the reaction product from 4-hydroxybenzoic acid with the authentic trimethylsilyl 4-trimethylsiloxybenzoate prepared by the method of Burkhard7) indicated that both carboxyl and hydroxyl groups were silylated and the yield was virtually quantitative.

Table I. Relative Retention Times of Trimethylsilyl Derivatives of Aromatic Acids on Silicone Columna)

Aromatic acid	Relative retention time		
	120°	140°	160°
Benzoic	0.22	0.15	
4-Hydroxybenzoic	1.91	1.00c)	1.00d)
4-Methoxybenzoic (anisic)	1.00b)	0.57	
3,4-Dihydroxybenzoic (protocatechuic)		2.80	2.42
3-Methoxy-4-hydroxybenzoic (vanillic)		2.03	1.85
3,4-Dimethoxybenzoic (veratric)		1.55	1.42
3,4,5-Trihydroxybenzoic (gallic)			4.80
Phenylacetic	0.29	0.19	
Mandelic	0.84	0.48	
Cinnamic	1.14	0.65	
Phenylpyruvic		1.51	1.44
Phthalic		1.40	1.33
Indoleacetic			3.44
Hippuric			2.53

a) Column size, 1/8 in.×6 ft. Column packing, 3% SE-52 on 60~80 mesh silanized Chromosorb W. N_2 flow rate, 30 ml. per min. Instrument, Perkin-Elmer Model 800.

b) Retention time for 4-methoxybenzoic acid at 120°, 16.8 min.

c) Retention time for 4-hydroxybenzoic acid at 140°, 12.4 min.

d) Retention time for 4-hydroxybenzoic acid at 160°, 5.1 min.

For separations of these TMS derivatives, we used preferentially silicone columns (SE-52 and XE-60). Polyester columns such as a diethylene glycol-succinate column were unsatisfactory for this purpose. While separations within a narrow range of molecular weight were effectively carried out under isothermal conditions, linear temperature-programmed procedure was successfully used for separations of mixtures with components of widely differing molecular weight.

Separations of nearly 40 aromatic acids were investigated in this study, and with most of the acids single, symmetrical peaks were obtained. Retention data of the acids having key struc-

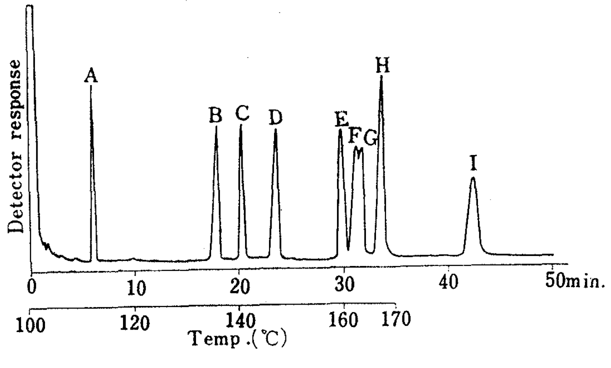

Fig. 1. Linear Temperature-Programmed Separation of Trimethylsilyl Derivatives of Hydroxy-substituted Benzoic Acids

Column and instrument as in Table I. Column temperature, programmed from 100° to 170° at a rate of 2° per min. Peaks: A, benzoic acid; B, 2-hydroxy-; C, 3-hydroxy-; D, 4-hydroxy-; E, 2,3-dihydroxy-; F, 2,6-dihydroxy-; G, 2,5-dihydroxy-; H, 3,4-dihydroxy, 2,4-dihydroxy-; I, 3,4,5-trihydroxy-.

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tures on a SE-52 column are shown in Table I. Phenolic acids and the corresponding methoxy acids (for example, protocatechuic and veratric) were well separated (Table I). Separation of positional isomers in hydroxy-substituted benzoic acids on silicone columns were generally satisfactory (Fig. 1).

It is anticipated that this extremely simple and rapid method for gas chromatography of aromatic acids will find extensive applications in biochemical and clinical research.

Faculty of Pharmaceutical Sciences,
Osaka University,
Toneyama, Toyonaka, Osaka

Tokushima Communication Hospital,
3 Iga-chō, Tokushima

Zen-ichi Horii (堀井善一)
Masami Makita (蒔田政見)
Ikuko Takeda (武田郁子)
Yasumitsu Tamura (田村恭光)
Yoshiaki Ohnishi (大西善明)

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