Table II. High Frequency Response of Some Bile Acids Dissolved in 60% Ethanol

Acid	Calibration Curve I				
	0.0001*M*	0.001*M*	0.002*M*	0.01*M*	0.02*M*
	Volt				
Taurocholic	0.79	7.6	14.9		
Glycocholic	0.40	2.77	4.23		
Cholic				0.92	1.51
Deoxycholic				0.97	1.63
Lithocholic				1.57	2.27
Dehydrocholic				1.26	2.00

acid is eluted with the front and glycodehydrocholic acid at 20–30–40 ml.

Figure 6 gives a separation of glycodeoxycholic acid at 10–15–20 ml. and glycolithocholic acid at 25–35–40 ml. The unconjugated lithocholic acid is eluted after the conjugates at 70–80–90 ml., but no recording was obtained.

For recording taurine conjugates with phase system D, the cell with the smallest sensitivity was used. A chromatogram of taurodeoxycholic and taurolithocholic acids is given in Figure 7.

DISCUSSION

The chromatograms show that the response depends on the nature of the substance, mainly as a function of the conductivity. The free bile acids have a dissociation constant of the order of 7×10^{-7} in 50% ethanol-water solution (*10*). The conductance of these acids is so low that they could not be detected in the effluent concentrations encountered (Table II). The conjugates are stronger acids. Their dissociation constants in water range from 10^{-3} to 10^{-4} (*8*). The bile acids with sulfonic substituents are the strongest acids. Thus it is possible to record taurine conjugates in much smaller concentrations than can be determined by titration. It is difficult to predict the conductivity of a compound in organic solvent systems because of the complex nature of these systems. In general, the acids and bases must have a dissociation constant of at least 10^{-5} in water to be detected by the method outlined.

The high frequency technique will find many applications in chromatographic work, especially in partition chromatography where organic solvents with low conductivity are used. The method is suitable for the analysis of substances with dissociation constants greater than 10^{-5}. Nonaqueous solvents may be used if the solvent mixture is such that ion pairs can be formed. The amount of salt or acid in the solvent must be kept low; otherwise the sensitivity will be decreased.

In this application, the demands on the stability of the high frequency apparatus are extremely high. Many precautions must be taken to avoid appreciable drift: Temperature change of the crystal will cause amplitude variations, and the tubes must be in excellent condition. Work is being carried out to improve the stability of the apparatus.

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Analysis of the Nonvolatile Acids in Cigarette Smoke by Gas Chromatography of Their Methyl Esters

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▶ The nonvolatile acid fraction of cigarette smoke, after conversion to a mixture of methyl esters with diazomethane, was analyzed by gas chromatographic techniques. Of the 16 esters detected, 11 have been identified. Lactic, glycolic, succinic, and malonic acids constitute about 75% of those identified, among which only succinic acid has been reported previously as a smoke constituent. Although some acids containing certain other functional groups cannot be analyzed by the techniques described, the method may be of value in the partial analysis of complex acid mixtures from sources other than cigarette smoke.

THE steam-volatile acids of cigarette smoke have been examined in detail (*4*), but little is known about the nonvolatile acids. In a survey of the literature up to 1954, Kosak (*18*) listed succinic, fumaric, citric, and phenolic acids as possibly present in cigarette smoke. Nicotinic and glutamic acids (*3*) and three α-keto acids (glyoxylic, pyruvic, and α-ketoglutaric) (*5*) have been detected since this report. The present paper is concerned with the detection and determination of a number of nonvolatile acids in cigarette smoke. The presence of succinic acid has been confirmed, whereas the other acids detected have not been reported previously.

Gas chromatography is the basis for the analytical method used. It is not

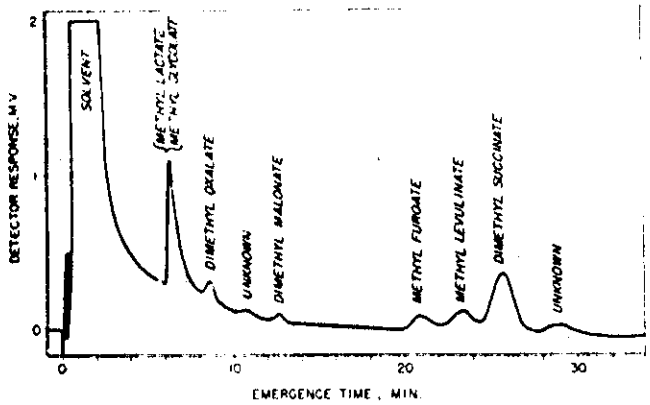

Figure 1. Gas chromatogram of smoke preparation at 138° C.

Sample, 20 μl. of methanol solution, equivalent to 0.4 cigarette. Column, 2 meters of dioctyl adipate on firebrick, 1 to 3 w./w. Helium flow, 17 ml./per min.

practicable to apply this technique directly to the separation of a mixture of the acids under consideration, because of their instability and lack of volatility. The methyl esters of these acids are readily examined by this method and, with a few exceptions, these esters can be obtained quantitatively by treating the mixture with diazomethane. Such a methylation step is an important feature of the analysis. This approach has also been taken in the analysis of fatty acids (*7, 10*), but with these acids direct gas chromatography has been successful (*7, 9*).

EXPERIMENTAL

Preparation of Smoke Sample. Fifty bright tobacco cigarettes without additives, 70 mm. long, were humidified over a saturated sodium bromide solution. These were smoked with an automatic smoking machine using one 35-ml. puff per minute of 2-second duration (*2*). Normally each cigarette was puffed 11 times, and the average butt length was 20 mm. The smoke was condensed in a series of six traps in dry ice–ethanol baths. The traps and connecting tubing were washed with 50 ml. of ether and then with five 20-ml. portions of 0.5% sodium hydroxide. The ether was extracted with each of the sodium hydroxide washes. The combined sodium hydroxide solution was extracted with three 50-ml. portions of ether and retained.

Isolation of Acids. Procedure A. Following the method of Resnik, Lee, and Powell (*13*), anions of organic acids in the sodium hydroxide extract were adsorbed on a 2 × 20 cm. column of Dowex-1 (CO_3^{--}). After washing with 200 ml. of water, the anions were removed with 400 ml. of 1.5*N* ammonium carbonate. In a modification of Resnik's procedure, excess ammonium carbonate in the eluent was destroyed by batch treatment with 300 grams of Dowex-50 (H^+), rather than by evaporation at 70° C. Supernatant liquid and six to eight 100-ml. water washes of the resin were passed through a 2.5 × 20 cm. column of Dowex-50 (H^+) to ensure complete conversion to free acids. Steam-volatile acids were removed by distillation at 20 to 30 mm. at a head temperature of 25° to 30° C., enough water being added to provide 1.5 to 2.0 liters of total distillate. Then 50 ml. of benzene were added and distilled to aid dehydration of the pot residue. The residue was dissolved in 50 ml. of 50% v./v. methanol-ether and held for methylation.

Procedure B. The sodium hydroxide extract was distilled to dryness at 25° to 30° C. at 20 to 30 mm. The residue was twice taken up in 300 ml. of water and the solution was distilled to dryness. This ensured removal of neutral and basic volatiles which might give extraneous peaks on a gas chromatogram. The residue was then taken up in 50 ml. of 4% acetic acid. Acids weaker than acetic were liberated; the stronger acids, formic and those classified as nonvolatile, remained in the salt form. Free acids were removed by extraction of the solution with five 50-ml. portions of ether. The aqueous solution was evaporated to dryness at 20 to 30 mm. Dehydration was completed by the addition and stripping of 50 ml. of benzene. The residue was dissolved in 100 ml. of 50% v./v. methanol-ether containing about 30 meq. of hydrogen chloride added to the solvent as the anhydrous gas. The precipitate of sodium chloride and other insolubles was removed by filtration and washed with 10 ml. of methanol. The filtrate was stripped at 20 to 30 mm. to about 10 ml., removing much of the excess hydrogen chloride which would later consume diazomethane. The solution then was made up to 50 ml. with the methanol-ether mixture. If a precipitate formed, sufficient methanol was added to ensure the dissolution of any organic acids therein.

Methylation of Acids. The solution of acids from either Procedure A or B was chilled in ice and treated with a moderate excess of diazomethane in ether. A precipitate of organic acids occasionally formed when the ether solution was added; this was redissolved by the addition of methanol, as methylation of suspended acids was impractically slow. After 2 hours, the solution was stripped to about 5 ml. and the exact volume was measured. The distillate was condensed in a dry ice trap and examined for diazomethane (yellow coloration). The residual solution was remethylated if no excess diazomethane appeared in this distillate. The diazomethane requirement is difficult to determine in advance; the amount usually added was about 0.03 to 0.05 mole. The analysis of the ester mixture should be completed a few days after its preparation, as on aging of the sample some changes in the gas chromatograms were noticed.

Preparation of Methyl Esters of Known Acids. For qualitative gas chromatographic studies, methyl esters of a number of known acids were prepared by adding diazomethane to a methanol-ether solution of 20 to 30 mg. of the acid. Excess diazomethane was stripped, and the residual solution was used directly for gas chromatography. This technique was successful for preparing the esters of most acids.

After an ester peak in a gas chromatogram of a smoke preparation had been identified, a larger quantity (0.5 to 1.0 gram) of the individual ester was prepared by methylation of the appropriate acid and purified by distillation. Solutions of definite concentrations of the known esters in methanol were prepared for quantitative gas chromatographic analysis. The solutions of all but two esters were usable over a period of several months. Dimethyl malate solutions failed to give an elution peak after standing a few weeks, while after aging a second peak appeared in methyl furoate solutions.

Qualitative Analysis of Methyl Esters. A Perkin-Elmer Vapor Fractometer Model 154-B with a Leeds & Northrup variable range recorder (Speedomax Type G) was used. The only modification of the Vapor Fractometer was the detachment of the solenoid valve from the vent line. The vent line was wrapped with a Nichrome heater to prevent condensation therein. Columns consisted of two glass U-tubes, 6 mm. in outer diameter and 1 meter long in series, or of a coil made from 3 meters of copper tubing, 1/4 inch in outer diameter. Useful stationary liquid phases are recorded in Table I. These were applied to Celite 545 (acid washed, 60 to 100 mesh) or C-22 firebrick (30 to 60 mesh) in a ratio of 1 to 3 w./w., except for the use of a 1 to 5 mixture with the viscous liquid II. Helium was used as carrier gas. The sample, injected into the gas stream with a hypodermic syringe, generally consisted of 20 to 40 μl. of a methanol solution containing a few milligrams per milliliter of the esters under consideration.

At a nominal temperature of 150° C., eight peaks were generally recognizable on chromatograms obtained from the use of columns with liquids I to VIII. These peaks represented esters having boiling points from that of methyl lactate (144° C.) to just above that of dimethyl succinate (193° C.). Figure 1 is a chromatogram of this type for a smoke sample treated by isolation Procedure A. The identity of the peaks was established by comparing their retention times on the different columns with those of known compounds under same conditions. Some typical results are given in Table II. It was not possible to effect a separation of methyl lactate and glycolate, comprising the first peak, with the columns used. However, with special columns of liquids IX and X at lower temperatures, this pair was successfully resolved (Table III).

At 190° C., esters having boiling points above that of dimethyl succinate and through that of dimethyl phthalate (282° C.) could be resolved. A typical chromatogram of a smoke sample treated by Procedure A is reproduced in Figure 2; another unknown and dimethyl phthalate were eluted at much longer retentions times and are not shown. Peak identification was accomplished using liquids I to III. Data are recorded in Table IV. No attempt was made to detect esters boiling higher than dimethyl phthalate.

Table I. Stationary Liquid Phases Used in Gas Chromatography of Methyl Esters

Designation	Description
	For 150° and 190° C. Separations
I	Flexol 8N8[a] [$(C_7H_{15}COOC_2H_4)_2NCOC_7H_{15}$]
II	Flexol R-2H[a] (polyester)
III	Flexol 4GO[a] (polyethylene glycol dioctanoate)
	For 150° C. Separations
IV	Flexol A-26[a] (dioctyl adipate)
V	Flexol TOF[a] (trioctyl phosphate)
VI	Tricresyl phosphate[b]
VII	Didecyl phthalate[b]
VIII	Dinonyl sebacate[c]
	For Lactate-Glycolate Separation[d]
IX	Carbowax 1500[a] (polyethylene glycol)
X	Carbowax 4000[a] (polyethylene glycol)

[a] Donated by Union Carbide Chemicals Co., New York, N. Y.
[b] Obtained from Eastman Kodak Co.
[c] Donated by Morton-Withers Chemical Co., Greensboro, N. C.
[d] Glycerol and diethylene glycol also permitted this separation but were too volatile at the required temperature.

Table III. Identification of Methyl Lactate and Glycolate

	Columns and Conditions			
Liquid phase[a]	Carbowax 4000		Carbowax 1500	
Solid support	Celite		Firebrick	
Length, m.	3		2	
Temp., °C.	123		122	
He flow, ml./min.	13		20	
	Retention Time, Min.			
	Known	Smoke	Known	Smoke
Methyl lactate	21.0	21.1	15.2	15.2
Methyl glycolate	29.0	28.9[b]	22.0	21.9[c]

[a] On solid support 1 to 3 w./w.
[b] Combined with dimethyl oxalate.
[c] Well separated from dimethyl oxalate peak at 19.5 minutes.

Table II. Identification of Methyl Esters by Comparative Gas Chromatography at 150° C.

	Columns and Conditions							
Liquid phase[a] Solid support	Dinonyl sebacate Firebrick		Didecyl phthalate Celite		Flexol 4GO Firebrick		Flexol A-26 Firebrick	
Length, m.	2		3		2		2	
Temp., °C.	154		146		146		138	
He flow, ml./min.	9		11		11		10	
	Retention Time, Min.							
Ester of	Known	Smoke	Known	Smoke	Known	Smoke	Known	Smoke
Lactic / Glycolic	7.0	7.1	6.9	6.9	5.0	5.0	6.0	6.0
Oxalic	8.2	8.1	11.4	11.5	7.2	7.2	8.7	8.6
Unknown	...	11.8	...	14.8	...	[b]	...	[b]
Malonic	13.3	13.1	17.1	17.3	11.8	11.6	13.9	13.8
Furoic	20.5	20.4	26.6	26.8	16.8	16.8	20.8	20.9
Levulinic	21.9	22.0	30.1	[c]	17.9	17.8	23.1	23.2
Succinic	23.5	23.8	30.3	30.7	19.6	19.7	25.6	25.6
Unknown	...	26.8	...	34.1	...	20.8	...	28.9

[a] On solid support 1 to 3 w./w.
[b] Not detected under these conditions.
[c] Not resolved from succinate peak.

Quantitative analysis. The same equipment was used as for the qualitative analysis. Samples of fixed volume, usually 20 µl., were injected into the apparatus with a Perkin-Elmer Micro-dipper pipet. Peaks on the chromatograms were enclosed by drawing a line connecting the base line just before and after the appearance of the peak; the areas enclosed were measured with a planimeter.

Columns and operating conditions were selected to give well resolved peaks in reasonable retention times. Those identified members of the ester preparation. A summary of conditions is given in Table V. Obviously, other combinations would also be practicable.

Calibration with standard solutions of an individual ester was performed in conjunction with an analysis at the same operating conditions. The solutions were of such concentration as to provide peak areas blanketing the area of the corresponding peak in the smoke preparation. Calibration curves, weight of ester plotted against peak area, were prepared when the relationship was not of direct proportion. The weight of ester giving a certain peak area in an analysis was determined. By employing an aliquot factor, the weight of the ester in the original smoke preparation was obtained.

Results of an analysis of a single smoke preparation are given in Table VI. The values are an average of two determinations, which generally agreed within 5 to 10%. Quantitative analysis was also performed on ester preparations from mixtures of known amounts of certain acids to determine recoveries in the various procedures. The results are recorded in Table VII. The acids in mixture I in methanol-ether solution

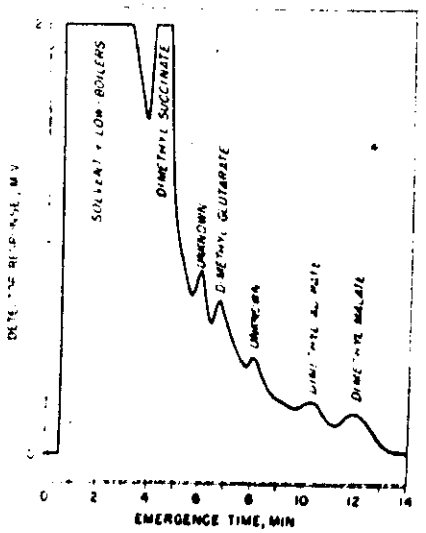

Figure 2. Gas chromatogram of smoke preparation at 190°C.

Sample, 20 µl. of methanol solution, equivalent to 1.8 cigarettes. Column, 2 meters Flexol R2H on firebrick, 1 to 3 w./w. Helium flow, ml./per min.

were treated with diazo[illegible] and stripped to a convenient volume. The acids in mixture II were dissolved in 0.5% sodium hydroxide and placed through isolation Procedure A and the methylation procedure. The acids in mixture III were dissolved in 0.5% sodium hydroxide and subjected to isolation Procedure B with subsequent methylation.

RESULTS AND DISCUSSION

Gas chromatography proved useful for the analysis of the nonvolatile acid fraction of cigarette smoke. The identification of 10 new acids (Table V) and the confirmation of succinic acid were possible by examination of this fraction after conversion to methyl esters. The identifications were made by comparing retention times of known esters with those of the peaks in a smoke preparation. Identification was considered conclusive when identity was established with each of three different columns. In many cases, checks on five to six columns were made. Five other peaks, not yet associated with specific compounds, were also noted. The specificity of the ion exchange method used in the sample preparation indicates that these result from additional acids rather than from other types of smoke constituents, but confirmation is lacking. Probably further acids are present in the smoke sample but were not detected. The acids reported may result, partly at least, from hydrolysis of esters in the smoke sample during the isolation procedures. Variations in the experimental conditions may cause differing extents of hydrolysis and thus influence the quantity of acids detected.

Both Celite and firebrick were satisfactory as solid supports, and the use of either one does not indicate any preference in a certain application. Those liquids used at 190° C. (stationary liquid phases) were sufficiently nonvolatile to permit sustained operations with the column for a few days, but prolonged use caused significant volatilization of the liquid.

The methyl esters chromatographed were eluted in the order of their boiling points, although substantial relative differences in retention times were noted from column to column. When two compounds were eluted with the same retention time on one column, their elution at different times was possible on another column—e.g., methyl levulinate and dimethyl succinate had essentially the same retention times on columns of liquids I, V, VI, and VII but different times with liquids III, IV, and VIII. Methyl lactate and glycolate mixtures were not resolved on any of the above columns; the more polar polyethylene glycols (liquids IX and X) did effect the separation of these two hydroxy compounds. Liquids IX and X also provided symmetrical elution peaks for these two compounds, whereas tailing peaks were obtained with most of the other columns. The separation of simpler alcohols on such columns has been reported (*1*).

Chromatograms of the smoke samples at 190° C. exhibited a rather slow return to the original base line, as seen in Figure 2. This may be due to partial degradation of this rather complex sample at high temperatures. This behavior was not encountered with samples of individual esters. There is a link with sample complexity, because the less pure product of isolation Procedure B gave a more extended tail on gas chromatography than that of Procedure A. This behavior is undesirable, but caused no difficulty in the qualitative work. In the quantitative analyses, conditions were sought which would provide a clean-cut peak on a flat base line for the compound under consideration.

During the identification work, experience was acquired with the methylation of other acids and with the chromatographic properties of their esters. Acids which underwent uncomplicated methylations and gave esters which could be readily chromatographed included citric, α-hydroxyisobutyric, and pimelic acids. Methylated salicylic acid gave only one peak, whose retention time suggested a boiling point nearer that of the methyl ester than of the dimethyl derivative; it is tentatively assumed that only carboxyl group methylation

Table IV. Identification of Methyl Esters by Comparative Gas Chromatography at 190° C.

	Columns and Conditions					
Liquid phase[a]	Flexol 8N8		Flexol 4GO		Flexol R-2H	
Liquid to solid, w./w.	1 to 3		1 to 3		1 to 5	
Temp., °C.	189		187		192	
He flow, ml./min.	18		18		8	
	Retention Time, Min.					
Ester of	Known	Smoke	Known	Smoke	Known	Smoke
Unknown	...	7.0	...	7.6	...	7.1
Glutaric	8.1	8.0	8.1	8.2	8.0	8.0
Unknown	...	9.4	...	9.4	...	9.6
Adipic	10.5	10.5	12.4	12.2	12.0	12.0
Malic	13.1	13.1	13.2	13.3	14.1	14.3
Unknown	...	17.8[b]	...	16.3[c]	...	11.7[d]
Phthalic	24.8[b]	25.5[b]	25.0[c]	25.8[c]	16.5[d]	16.7[d]

[a] On firebrick in glass coilfmns.
[b] At He flow 34 ml./min.
[c] At He flow 38 ml./min.
[d] At He flow 26 ml./min.

Table V. Some General Conditions for Quantitative Determination of Esters by Gas Chromatography

Methyl Ester of	Column		Temp., °C.	Helium, Ml. per Min.
	Liquid[a]	Length, m.		
Glycolic	IX	3	120	20
Lactic	X[b]	3	100	13
Oxalic	VII	3	110	13
Malonic	IV	2	115	13
Furoic, Levulinic, Succinic	IV	2	130	13
Glutaric	II[c]	3	170	13
Adipic, Malic	II[c]	2	185	8
Phthalic	II[c]	2	185	26

[a] On firebrick, 1 to 3 w./w.
[b] On Celite, 1 to 3 w./w.
[c] On firebrick, 1 to 5 w./w.

Table VI. Analysis for Identified Nonvolatile Acids in Bright Tobacco Cigarette Smoke

Acid[a]	Mg./100 Cigs.[b]	Meq./100 Cigs.[b]
Lactic	30	0.33
Glycolic	29	0.38
Succinic	19	0.32
Malonic	9	0.2
Furoic	6	0.05
Malic[c]	6	0.08
Phthalic[c]	4	0.05
Oxalic	4	0.08
Levulinic	3	0.03
Glutaric[c]	3	0.04
Adipic[c]	0.6	0.008

[a] Isolated by Procedure B.
[b] Values subject to revision with isolation procedures giving more complete recoveries.
[c] Isolated by Procedure A.

Table VII. Analysis of Known Mixtures of Acids

Acid	Mixture I[a] Mmole charged	Mixture I[a] Mmole recovered	Mixture I[a] % recovery	Mixture II[b] Mmole charged	Mixture II[b] Mmole recovered	Mixture II[b] % recovery	Mixture III[c] Mmole charged	Mixture III[c] Mmole recovered	Mixture III[c] % recovery
Isobutyric	0	...	...	0	...	...	0.78	0[d]	0
Furoic	0.062	0.060	97	0.15	0	0	0.16	0.085	53
Heptylic	0	...	...	0	...	...	0.43	0[d]	0
Lactic	0.31	0.30	97	0.48	0.21	44	0.60	0.59	98
Malonic	0	...	...	0	...	...	0.37	0.36	97
Oxalic	0.094	0.091	97	0.22	0.10	46	0.18	0.10	56
Succinic	0.31	0.30	97	0.39	0.25	64	0.51	0.49	91

[a] Treated directly with diazomethane.
[b] Isolation Procedure A and reaction with diazomethane.
[c] Isolation Procedure B and reaction with diazomethane.
[d] Quantitatively removed in isolation procedure.

occurred under the conditions employed. Methylated oxalacetic and pyruvic acids each gave single peaks at retention times in line with the boiling points of their simple methyl esters. However, the possibility of interaction of the carbonyl group and diazomethane (*6*) producing some other substance cannot be ignored. With methylated glyoxylic acid, two large peaks were obtained whose identity has not been explored. With the smoke constituent levulinic acid, no interference by reaction of the carbonyl group occurred. The methylated product which was isolated boiled at the correct temperature for methyl levulinate. Maleic and fumaric acids gave peaks only when carefully methylated; when excess diazomethane was added, no peaks were obtained. This is probably due to formation of a nonvolatile pyrasoline by reaction of the double bond with diazomethane (*8*). Two amino acids, glycine and nicotinic acid, were methylated but neither gave chromatographic peaks, possibly because of the formation of nonvolatile products through interactions with the aminoid nitrogen. The latter compound produced a brown tar with diazomethane. Tartaric and α-ketoglutaric acids consumed diazomethane, but no peaks could be obtained on gas chromatography of the products. Other conditions may lead to their successful elution.

Some of the difficulties with diazomethane may be overcome by employing other methylation techniques. The presence of additional nonvolatile acids in cigarette smoke may then be observed. Other isolation procedures designed to avoid destruction of highly reactive substances, such as pyruvic and glyoxylic acids, may also lead to the detection of additional acids.

Quantitative determinations with gas chromatography were based on the relationship of peak area to the weight of the ester giving this peak. The minimum area usually was about 1 to 2 sq. cm. Instrumental conditions were set so that about 20 γ of an ester gave this area. The concentrations of most of the esters in a smoke preparation gave considerably larger areas. Calibrations revealed that area-weight relationships varied from direct proportionality to slight nonlinearity. The phenomenon of a nonlinear area-weight relationship has been observed previously (*11*).

The values reported in Table VI are those actually found and are not corrected for losses which occurred in the isolation procedures. Usually the accuracy was better than 90%. Notable exceptions are furoic and oxalic acids, whose actual values may be almost double those in Table VI.

In the analysis of a known mixture of four typical acids subjected to the methylation procedure, recoveries of 97% were obtained (Table VII). Isolation Procedure A did not give quantitative recoveries of nonvolatile acids in a few tested cases. In Table VII, recoveries in the analysis of a representative known mixture were only about 50%; no recovery was effected for furoic acid. The loss occurs in the removal by steam distillation of the volatile acids; the simpler nonvolatile acids are actually slightly volatile, and with the small quantities involved, a significant loss occurred. Procedure B, which utilizes differences in acid strength as the basis for separation, avoids any distillation of aqueous solutions of the acids and performs the separation desired (Table VII). Recoveries of lactic, malonic, and succinic acids were excellent. Substantial losses of furoic and oxalic acids were still encountered and further improvement of the isolation scheme is desired.

Isolation Procedure B provides no clear-cut separation of the acids from all other smoke constituents, as is possible with Procedure A with its ion exchange features. Consequently, the final methylated samples are considerably more complex. Nevertheless, gas chromatograms at 150° C. of the final samples from either procedure were qualitatively almost identical. As the product of Procedure B gave chromatograms at 190° C. which indicated sample degradation, this procedure is best applied to the analysis of acids whose esters have the lower boiling points. Procedure A is probably satisfactory for the acids of high boiling esters because their acids should not be as subject to loss in the distillation step, although this has not been established. The analysis of smoke acids in Table VI is based nevertheless on this approach. A combination of the ion exchange features of Procedure A and the separation features of Procedure B should lead to a clean sample with good recovery of the desired acids.

The application of gas chromatography to the determination of the common nonvolatile organic acids has not been reported previously. It represents a useful addition to the other chromatographic methods available to the analyst concerned with this problem. Some acids studied and others of a related nature commonly occur in biological systems and products. The gas chromatographic methods described should be of value in the study of these acids from other sources.

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