Separation of Some Tobacco Alkaloids by Gas Chromatography

GAS chromatography has been employed in separating numerous types of compounds, but has not yet been applied to the alkaloids, a field where it might find particular utility. We wish to report preliminary results clearly indicating the usefulness of gas chromatography in the study of the tobacco alkaloids. Other families of alkaloids having slight volatility and good thermal stability may also prove to be separable under similar conditions.

For the stationary liquid phase we found certain polyglycols (Table 1) preferable to the less-polar materials, such as 'Apiezon' grease and the silicones, commonly used in high temperature work. The stability and non-volatility of the polyglycols are sufficient to permit continuous use of columns for several days without appreciable changes in their retention ability. The alkaloids were eluted in sharp, symmetrical peaks with columns containing the polyglycols.

Table 1

	Columns and conditions		
Liquid phase	Polyethylene glycol (*a*)	Polypropylene glycol (*b*)	Polybutylene glycol (*c*)
Temperature	190° C.	190° C.	180° C.
Helium flow (ml./min.)	48	45	50
	Retention time (min.)		
3-Pyridyl methyl ketone	4·3	4·3	3·1
3-Pyridyl ethyl ketone	5·3	6·1	5·0
3-Pyridyl *n*-propyl ketone	6·6	8·1	7·0
Nicotine	5·2	8·6	8·2
Nor-nicotine	12·3	16·1	14·3
Myosmine	13·4	16·4	14·7
Anabasine	13·8	19·4	18·1
Metanicotine	16·5	23·5	20·9
Nicotyrine	19·4	21·0	18·3
Cotinine	85	79	63

(*a*) Molecular weight, 20,000 (Dow Chemical Co.). (*b*) Molecular weight, 1,025 (Union Carbide Chemical Co.). (*c*) Molecular weight, 1,500 (Dow Chemical Co.).

The polyglycol columns exhibited good selectivity for the alkaloids, making possible the separation of most of the members of complex mixtures. This is seen from the retention-time data in Table 1. The elution sequence is identical on the polypropylene and polybutylene columns, although some relative differences in retention times are observable. A slightly different sequence was noted for the polyethylene glycol column. These effects are valuable in that a separation of two alkaloids difficult on one column may be feasible on another.

That the structure of the alkaloids had not been altered by the rather severe conditions of the gas chromatographic technique was established by obtaining ultra-violet absorption spectra for samples condensed from the gas stream as the elution peak for an alkaloid, run individually, was being recorded. In every case, the collected and starting compounds were identical. Paper chromatographic studies, using solvent systems *S*1 and *L*5 of Kuffner *et al.*[1], were also performed in some cases and verified this conclusion.

The Perkin–Elmer Vapor Fractometer, Model 154–*B*, modified to include a thermostat for the column chamber and a heater on the vent line from this chamber, was used in this study. Samples were 20λ aliquots of benzene solutions containing 5–10 mgm. per ml. of the alkaloids. Helium was used as carrier gas. The solid support was alkali-washed firebrick; liquid phases were placed thereon in the weight ratio of 1 to 4. Columns were U-tubes of 6 mm. outside diameter glass tubing, 1 m. long. The helium flow was determined at the vent with a soap-bubble meter at room temperature.

The alkaloid fraction of cigarette smoke is being investigated with the techniques described here. Results will be published in a subsequent paper.

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[1] Kuffner, Schick and Bühn, *Monatsh.*, **87**, 749 (1956).

Complex Phospholipids

RECENT work has revealed the existence of hitherto unsuspected phospholipids in addition to the three major components, lecithin, phosphatidyl ethanolamine and phosphatidyl serine, and two minor components, sphingomyelin and phospho-inositide. Lea, Rhodes and Stoll[1], by chromatography on silicic acid, obtained lysolecithin and lysocephalin from egg yolk phospholipids, and Marinetti, Erbland and Kochen[2] have shown by paper chromatography that unidentified phospholipids were present in a number of tissues. This work is supported by the results obtained by Dawson[3] and Benson and Maruo[4]. Collins and Wheeldon[5], working with the N-2 : 4-dinitrophenylated and methylated derivatives of the phospholipids from rat and sheep brain and rat liver, have shown the presence of a fraction of the ethanolamine- and serine-containing phospholipids which corresponds neither to phosphatidyl ethanolamine nor to phosphatidyl serine. The present work is concerned with the presence of additional new and as yet unidentified compounds in this fraction.

Rat liver lipids, after N-2 : 4-dinitrophenylation and methylation as described by Collins and Wheeldon[5], were separated into polar and non-polar fractions by counter-current distribution in 85 per cent (v/v) aqueous ethanol-light petroleum. The non-polar fraction was further fractionated by chromatography on 'Hyflo-Supercel' (1 mgm. lipid to 2 gm. of adsorbent) and four materials containing phosphorus separated by elution with the solvent sequence, light petroleum, light petroleum-benzene mixtures, benzene, benzene–chloroform mixtures and chloroform. The characteristics of these four fractions are shown in Table 1.

Chromatography of the polar fraction led to its decomposition, but counter-current distribution enabled the isolation of three dinitrophenyl-containing compounds and certain of their properties have been determined (Table 2). One of these compounds has been isolated in sufficient quantity for a full analysis and appeared to be homogenous as shown by the distribution curve. Sheep brain lipids were found to be the best source for its isolation.

Determination of the molecular weight by an isopiestic method[6] and by the depression of the freezing point in cyclohexane indicated values of