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THE CONSTITUENTS OF CERTAIN TOBACCO TYPES—I.

STEAM VOLATILE PHENOLS OF LATAKIA

W. J. IRVINE and M. J. SAXBY

Carreras Ltd., Research Division, Nevendon Road, Basildon, Essex

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Abstract—A phenolic extract of Latakia tobacco was steam distilled and resolved into a number of components by means of thin-layer and gas chromatography. By comparison with authentic compounds, twenty monohydroxylic phenols have been detected.

INTRODUCTION

LATAKIA leaf is widely used in the pipe-tobacco industry and yet the chemical components responsible for its characteristic odour and flavour have received scant attention. The leaves of Latakia tobacco are small and are generally cured in smoke houses in which the smoke from burning pine or oak brushwood is allowed to permeate the leaf, over a period of several months. Originally coming only from Syria, much Latakia tobacco is now produced in Cyprus.

Although little information exists regarding the chemical composition of Latakia leaf, reports have been made on the constituents of wood smoke. Special regard has been paid to the phenols of Hickory Sawdust Smoke and their relation for the food industry.[1]

Thin-layer chromatography (TLC) and gas–liquid chromatography (GLC) provide excellent methods for the separation and identification of phenols. After conversion to their azodyes with *p*-nitraniline, many phenols are readily separated by reverse phase TLC.[2] Using GLC methods free phenols may be separated without "tailing effects" on deactivated columns, whereas the corresponding acetates may be chromatographed on columns using trixylenyl phosphate[3] and Carbowax as stationary phases.

RESULTS AND DISCUSSION

A phenolic fraction obtained by sodium hydroxide extraction of the ether-soluble portion of Latakia tobacco was steam distilled. After extraction, drying and careful evaporation of solvent, the residue was analysed by TLC and GLC.

A dye mixture prepared from this residue and diazotized *p*-nitraniline was applied to kieselguhr plates impregnated with formamide (10% w/v in acetone). After development in solvent 1 (Table 1) twelve spots were observed, of which two were highly coloured and could readily be identified as phenol (red) and *m*-cresol (purple). By the use of authentic compounds,

[1] W. FIDDLER, R. C. DOERR, A. E. WASSERMAN and J. M. SALAY, *J. Agr. Food Chem.* **14**, 659 (1966).
[2] G. A. L. SMITH and P. J. SULLIVAN, *Analyst* **89**, 312 (1964).
[3] V. T. BROOKS, *Chem. Ind.* 1317 (1959).

eight of the spots were tentatively identified, whilst phenols substituted in the *para* position migrated to the top of the plate. Certain pairs of phenols travelled at the same rate on the chromatogram and thus could not be resolved, so that positive identification could only be given to *o*-cresol 2,6-xylenol and *m*-methoxyphenol. No resolution took place between the pairs of compounds 3,5-xylenol and *o*-ethylphenol; 2,5-xylenol and *o*-ethylphenol; guaiacol and 2,6-dimethoxyphenol.

TABLE 1. PHENOLIC COMPOUNDS DETECTED IN LATAKIA

	Thin-layer chromatography of azo dyes on formamide-treated kieselguhr					Gas–liquid chromatography of compounds and their acetates		
	Solvents*					Column†		
	1	2	3	4	Azodye λ_{max}	1	2	3
Phenol	R_p value		R_c value		(nm)	retention time relative to Phenol	retention time relative to Phenyl acetate	
Phenol	1·00	1·00	—	—	498	(1·00)	1·00	1·00
o-Cresol	1·93	2·00	—	—	514	1·14	1·24	1·25
m-Cresol	1·77	1·69	—	—	510	(1·64)	(1·47)	1·41
p-Cresol	—	—	1·00	1·00		(1·64)	(1·47)	1·50
2,3-Xylenol	—	(3·66)	—	—		2·26	2·47	3·15
2,4-Xylenol	—	—	0·48	1·67		(1·77)	(1·82)	(2·20)
2,5-Xylenol	(2·60)	—	—	—	526	(1·64)	1·70	(2·20)
2,6-Xylenol	1·30	(3·66)	—	—		(1·00)	(1·47)	1·70
3,5-Xylenol	3·00	(2·34)	—	—		(2·58)	(2·00)	2·02
m-Ethylphenol	(2·60)	(2·34)	—	—	513	(2·58)	(1·82)	(2·20)
p-Ethylphenol	—	—	(0·42)	1·75		(2·58)	(2·00)	2·42
Guaiacol	(0·33)	(3·66)	—	—		0·68	3·30	3·35
m-Methoxyphenol	0·20	0·33	—	—		0·615	3·94	4·15
p-Methoxyphenol	—	—	0·22	1·22		5·14	4·23	4·65
4-Methylguaicol	—	—	—	—		1·20	—	(5·75)
4-Ethylguaiacol	—	—	—	—		(1·77)	(6·00)	9·80
4-Propylguaiacol	—	—	—	—		(2.58)	7·65	(5·75)
4-Vinylguaiacol	—	—	—	—		(1·77)	(6·00)	8·55
Eugenol	—	—	—	—		3·01	9·40	12·50
2,6-Dimethoxyphenol	(0·33)	1·23	—	—		4·56	11·10	14·75

* Solvents: 1, benzene–cyclohexane–diethylamine, 5:5:1; 2, benzene–cyclohexane–dipropylene glycol, 30:70:3; 3, cyclohexane–diethylamine, 9:1; 4, cyclohexane–benzene–diethylamine, 7:3:1.
Figures in brackets show unresolved spots or peaks:

$R_p = R_f$ relative to azo dye from phenol.
$R_c = R_f$ relative to azo dye from *p*-cresol.

† Column 1, 3% neopentylglycol adipate at 125°; 2, 15% carbowax 20 M at 165°; 3, 5% trixylenylphosphate at 135°.

The dye mixture was further chromatographed on a kieselguhr plate impregnated with formamide using solvent 2 for development. In this solvent, spots were visualized with ammonia vapour and, with the aid of authentic compounds, tentative identifications were made. As in the previous chromatogram, certain phenols could not be resolved. Authentic samples of 2,5-xylenol and *o*-ethylphenol azodyes on chromatographing in solvent system 2 had R_p values of 3·11 and 3·46 respectively, but were not located in the distillate extract.

Although 2,5-xylenol was later found by GLC, its concentration was too low for detection by TLC.

In both solvents 1 and 2 the *para*-substituted phenols were located close to the solvent front as a bright yellow band, which was removed from the plate and reapplied to a further formamide-impregnated kieselguhr plate. After development in solvent 3 (Table 1), four spots were located, which by comparison with authentic compounds were tentatively identified. In this system, *p*-ethylphenol and 3,4-xylenol samples were coincident, but good separation occurred in the solvent mixture cyclohexane/benzene/diethylamine (7:3:1) (solvent 4, Table 1). 3,4-xylenol was not located in the mixture, since an authentic sample did not coincide with any spots on the chromatogram.

After development in solvent 1 (Table 1) the most intense azodyes were removed, dissolved in glacial acetic acid, centrifuged to remove kieselguhr and their visible absorption spectra determined. These absorption maxima were compared with some authentic dyes to aid identification, and thus confirms that in solvent 1, *m*-ethylphenol rather than 2,5 xylenol, is the main component of the spot of R_p 2·60.

An attempt was also made to separate free phenols by GLC on a 4 m column of Apiezon L,[4] but the degree of separation of some authentic compounds was insufficient for the purposes of this investigation.

Accordingly, a portion of steam distillate was analysed by GLC on a column of 3% neopentylglycol adipate partially deactivated with trimer acid operating at 125° (column 1, Table 1).

Using the column of neopentylglycol adipate, 3,4-xylenol possessed a relative retention time of 2·97 and did not correspond to any peak in the chromatogram. A number of unknown compounds were also found, all of low intensity. Although the four phenols of relative retention 2·58 (Table 1) had slightly differing retention times when chromatographed individually, a given synthetic mixture of the four could not be resolved. Isoeugenol yielded a relative retention time of 6·92, not identical to any peaks in the chromatogram.

A further sample of the dry steam distillate was acetylated and injected onto a column of 15% Carbowax 20 M operating at 165°. Use of the phenyl acetates rather than the free phenols resulted in somewhat better separation of isomers and other closely related phenols.

Further verification of the identity of phenols present in the steam distillate was obtained by injection of the acetate mixture together with authentic compounds onto a column 5% trixylenyl phosphate partially deactivated with 1% phosphoric acid, operating at a temperature of 135°.

Using the column of trixylenylphosphate, 3,4-xylenyl acetate had a retention time close to the acetate of 2,3-xylenol, but its presence had been excluded by the results from the chromatography of the free phenols.

The separation and positive identification of individual xylenols and ethyl phenols presents a considerable problem. It has not been possible to separate all isomers and other closely related phenols by every method, but all the compounds listed have been separated unambiguously by at least one procedure. Using reverse phase TLC *o*-ethylphenol could not be detected, but its presence in a low concentration cannot be totally excluded since an adequate separation from phenols known to be present could not be achieved by GLC. Almost all the phenols found in Latakia tobacco may be adequately separated by GLC when present in approximately equal quantities in artificial mixtures, but separation becomes impossible when a few compounds are present in excess. Unfortunately, conditions of this

[4] W. NAUCKE and F. TARKMANN, *Brennstoff-Chem.* **45**, 263 (1964).

nature often occur in natural products and have prevented the identification of many important minor components. Many workers have collected effluents from gas chromatographs and compared their i.r. spectra with authentic compounds,[5] using in particular the "finger print" region for confirmation. However, even a small quantity of impurity arising from some previous large peak can produce marked changes in the i.r. spectrum in the region 700–1400 cm^{-1} so confusing their identification.

Except in very qualitative terms, it is very difficult to gauge the concentrations of the phenols in the distillate. No attempt was made to extract compounds quantitatively from the leaf and there is no certainty that the distillation stage removed all steam volatile phenols equally. However, it would appear that phenol, 4-vinylguaiacol, *m*- and *p*-cresols are the major components and that moderate quantities of 2,6-dimethoxyphenol, eugenol, *o*-cresol, 2,3-, 3,5- and 2,4-xylenols are present.

Samples of 2,3,5-trimethylphenol and 2,3,5,6-tetramethylphenol were available and when chromatographed in the free state on the neopentylglycol adipate column, they possessed retention times closely corresponding to two unknown peaks. However, after conversion to their acetates, corresponding peaks on the other two columns could not be found and it is assumed that they are absent despite their volatility in steam.

There can be little doubt that many of the phenols listed in Table 1 play an important role in the odour of Latakia tobacco. An organoleptic evaluation of guaiacol, 4-methylguaiacol and 2,6-dimethoxyphenol in wood smoke has recently been made and their importance has been discussed.[6] The presence of 4-methylguaiacol and eugenol in Jamaica Rum[7] has recently been reported and it has been known for more than a decade that 4-ethylguaiacol is an important flavour constituent of Soy sauce.[8]

EXPERIMENTAL

Preparation of Extract

Ground Cyprus Latakia tobacco (1·5 kg: moisture = 10·1 %) was thoroughly extracted with light petroleum (60–80°) in a large Soxhlet extractor to remove waxes.[9] After brief drying, the tobacco was further extracted with ether until no more pigment was apparently removed. The ethereal solution was carefully evaporated under slightly reduced pressure to about 2 l. and then repeatedly extracted with a saturated aqueous solution of sodium bisulphite. The ethereal solution was thoroughly washed and re-extracted with 2 N H_2SO_4. This procedure was repeated with sodium hydrogen carbonate solution and finally 2 N sodium hydroxide solution. The final alkaline extract was acidified to Congo Red and extracted into ether which, after drying and evaporation, yielded a thick dark residue (68 g). Portions of this residue (3–4 g) were steam distilled just prior to use for TLC or GLC.

Identification of Compounds

Authentic compounds used during this investigation were either purchased from chemical supply houses or synthesized from available starting materials.

Reduction of vanillin with Zn amalgam in HCl yielded 4-methylguaiacol,[10] b.p. 123–124°/25 mm. A similar reduction of acetovanillone gave 4-ethylguaiacol,[11] b.p. 146°/25 mm. Catalytic hydrogenation of eugenol yielded 4-*n*-propylguaiacol,[12] b.p. 158°/25 mm. Pyrolysis of ferulic acid in the presence of copper powder produced the required 4-vinylguaiacol,[12] b.p. 136°/25 mm.

[5] I. SCHMELTZ, C. D. STILLS, W. J. CHAMBERLAIN and R. L. STEDMAN, *Anal. Chem.* **37**, 1614 (1965).
[6] A. WASSERMAN, *J. Food Sci.* **31**, 1005 (1966).
[7] M. MAARSE and M. C. DE BRAW, *J. Food Sci.* **31**, 951 (1966).
[8] T. YOKOTSUKA, *J. Agr. Chem. Soc. Japan* **27**, 276 (1953).
[9] R. N. GLADDING and H. E. WRIGHT, *Tobacco Sci.* **3**, 81 (1959).
[10] R. SCHWARZ and H. HERING, *Org. Syn.* **33**, 17 (1953).
[11] T. YOKOTSUKA, *J. Agr. Chem. Soc. Japan* **27**, 276 (1953).
[12] C. J. COSCIA, W. J. SCHUBERT and F. F. NORD, *J. Org. Chem.* **26**, 5085 (1961).

Gas Chromatography

All columns were of stainless steel and of dimensions 2 m × 0·32 cm (o.d.) and in all experiments a flame ionization detector was employed.

Free phenols were separated on a column of neopentylglycol adipate (3%) containing a trimer acid (0·75%) on Chromosorb W (80–100 mesh) pretreated with hexamethyldisilazane (HMDS). The column was operated at 125° with an injection temperature of 200° and a nitrogen gas flow rate of 22 ml/min at a pressure of 3·2 kg/cm^2.

Phenols were extracted from the steam distillate with ether, washed with $NaHCO_3$ solution, dried and evaporated to small bulk. 1 μl quantities of the extract were injected onto the column and peaks identified by comparison of retention times with authentic compounds.

Phenyl acetates were separated on two phases. A column of trixylenyl phosphate (5%) and phosphoric acid (1%) on HMDS Chromosorb W (80–100 mesh) was operated at 135° with an injection temperature of 200°. The gas flow rate was adjusted to 19 ml/min at 3·2 kg/cm^2.

A second column of Carbowax 20 M (15%) on Chromosorb P (60–80 mesh) was operated at 165° with an injection temperature of 200° and a flow rate of 22 ml/min at 3·2 kg/cm.[2]

Acetates were prepared by shaking a strongly alkaline solution of the distillate with acetic anhydride followed by extraction of the acetates into ether. The ethereal solution was washed three times with $NaHCO_3$ solution, dried and evaportated to low bulk. The residue was used for injection into the chromatograph.

Thin-layer Chromatography

A slurry of kieselguhr G with water was spread on 20 × 20 cm glass plates to a thickness of 0·25 mm. Plates were dried for 0·5 hr at 100° and cooled. Immediately before use the plates were impregnated with a 10% solution of formamide in acetone and allowed to dry at room temperature for 10 min.

Azodyes of the phenols were prepared by adding cold $NaNO_2$ solution (0·2%) to *p*-nitraniline (0·1%) in 0·2 N HCl until colourless. The distillate (or a solution of an authentic compound) was adjusted to Hp 10 with dilute NaOH solution and the two mixtures combined to produce the azodye. After acidification with 2 N HCl, the dye was extracted into ether, dried and evaporated to 1 ml.

Azodye mixtures were spotted onto the formamide-impregnated plates and developed by allowing the solvent to run 12 cm. Compounds were identified by running simultaneous samples of authentic azodyes.

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