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FURTHER VOLATILE PHENOLS OF LATAKIA TOBACCO LEAF*

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Abstract—The phenolic extract of Latakia tobacco was analysed by conversion of the phenols to their corresponding acetates and separation of the components by capillary gas chromatography. Thirty-eight phenols have been identified by comparison with authentic compounds on three stationary phases.

INTRODUCTION

IN A PREVIOUS publication,[1] twenty steam-volatile phenols from Latakia tobacco were identified by use of packed column GLC and TLC. As a natural extension of this work it was decided to investigate some further phenols of the Latakia extract.

The low volatility of the phenols under investigation necessitated their conversion to suitable derivatives before gas chromatography. Langer *et al.*[2] suggested the conversion of phenols to their corresponding trimethylsilyl ethers, whilst Smith and King[3] used the methyl ether and acetyl derivatives in a study of the steam-volatile phenols in cigarette smoke by capillary column GLC. The separation of a large number of methyl-substituted phenols as their trifluoracetate esters is reported by Shulgin.[4] In this investigation the phenols were converted to the corresponding acetates before chromatography.

RESULTS AND DISCUSSION

A quantity of Latakia tobacco was extracted with ether and a phenolic fraction obtained from it by extraction with aqueous NaOH. A portion of this fraction was acetylated and the mixture of phenyl acetates analysed on three capillary columns coated with polyphenyl ether OS124 (PPE), trixylenyl phosphate (TXP) and diethylene glycol succinate (DEGS) respectively. Unknown peaks were identified by comparison of their relative retention times with those of authentic compounds.

The DEGS support-coated column separated the extract into thirty-five peaks, of which twenty-six were identified by comparison of retention times. A series of phenyl acetates was chromatographed but these were not found in the mixture. These included the acetates of resorcinol (relative retention time 1·19), 3-methylcatechol (1·21), orcinol (1·50), 2-methylresorcinol (1·32), methylquinol (1·49) 2,4,6-trimethylphenol (0·29), 1,2,5,6-tetramethylphenol (0·83), and *o*-allylphenol.

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Certain pairs of compounds gave coincident retention values on one stationary phase, but were separated on the other columns. Since the acetates of 2,6-xylenol and *o*-ethylphenol were unresolved on all three stationary phases, and the presence or absence of *o*-ethylphenol was not established in the earlier work, it was necessary to prepare a suitable derivative of

TABLE 1. PHENOLS DETECTED IN LATAKIA TOBACCO LEAF

Phenol (acetate)	Relative retention times on		
	DEGS	PPE	TXP
Phenol	0·13	0·17	0·16
o-Cresol	0·16	0·23	0·21
m-Cresol	0·19	0·28	0·24
p-Cresol	0·19	0·29	0·26
2,3-Xylenol	0·32	0·46	0·44
2,4-Xylenol	0·25	0·37	0·34
2,5-Xylenol	0·23	0·39	0·34
2,6-Xylenol	0·19	0·33	0·30
3,4-Xylenol	0·34	0·55	0·50
3,5-Xylenol	0·28	0·46	0·40
o-Ethylphenol	0·19	0·33	0·30
m-Ethylphenol	0·28	0·41	0·34
p-Ethylphenol	0·26	0·44	0·40
2-*n*-Propylphenol	0·28	0·44	0·40
4-*n*-Propylphenol	0·39	0·64	0·60
2,3,5-Trimethylphenol	0·41	0·72	0·57
2,3,6-Trimethylphenol	0·41	0·44	0·60
2,4,5-Trimethylphenol	0·48	0·72	0·63
3,4,5-Trimethylphenol	0·60	1·04	0·83
Guaiacol	0·46	0·55	0·54
3-Methoxyphenol	0·54	0·68	0·62
4-Methoxyphenol	0·60	0·72	0·71
4-Methylguaiacol	0·65	0·83	0·78
5-Methylguaiacol	0·62	0·83	0·59
4-Ethylguaiacol	0·75	1·09	1·00
4-Propylguaiacol	0·87	1·39	1·25
4-Vinylguaiacol	0·73	1·08	1·04
2,6-Dimethoxyphenol	1·25	1·44	1·35
2,3-Dimethoxyphenol	0·93	1·04	1·04
Catechol	1·00	1·00	1·00
Quinol	1·25	1·29	1·21
Eugenol	1·00	1·46	1·35
Isoeugenol	1·15	1·65	1·47
	1·47	2·12	1·95
4-Methylcatechol	1·25	1·39	1·35
4-Methyl-2,6-dimethoxyphenol	1·47	1·86	1·70
4-Ethyl-2,3-dimethoxyphenol	1·71	2·19	1·97
3,5-Dimethoxyphenol	1·47	1·72	1·61
2-Methoxycatechol	2·36	2·05	—

this pair which could be separated by GLC. Accordingly a portion of the extract was methylated and the anisole derivatives chromatographed on Apiezon L. Peaks corresponding to 2,6-dimethylanisole (absolute retention time 3·7 min) and *o*-ethylanisole (5·3 min) were identified.

All the compounds identified on the DEGS stationary phase were confirmed on the PPE and TXP surface-coated columns. The PPE column resolved the mixture into forty peaks of

which twenty-eight were identified, while the TXP column produced thirty-nine peaks of which twenty-six were identified, some of which consisted of coincident pairs of compounds.

Eighteen of the thirty-eight phenols have been identified in Latakia tobacco for the first time. The remainder were previously identified in the steam distillate of the phenolic extract.[1] A number of phenols identified in the extract have not been found before in either tobacco or tobacco smoke. These are 2-*n*-propylphenol, 4-*n*-propylphenol, 2,3,6-trimethylphenol, 2,4,5-trimethylphenol, 3,4,5-trimethylphenol, 5-methylguaicol, 2-methoxycatechol, 2,3-dimehtoxyphenol, 4-methyl-2,6-dimethoxyphenol, 3,5-dimethoxyphenol and 4-methyl-catechol. 2-Methoxycatechol and 4-methyl-2,6-dimethoxyphenol are, however, known constituents of wood tar,[5] while 2,3-dimethoxyphenol has been identified in meat-curing smoke.[6]

In addition to the trimethylphenols mentioned above, 2,3,5-trimethylphenol was also identified in the extract, but this has previously been reported as a constituent of tobacco smoke.[3,7] 2,4,6-Trimethylphenol, which has been previously found in tobacco smoke,[7] was not identified in the Latakia extract. Despite the absence of resorcinol in the mixture, both catechol and quinol, together with 2-methoxycatechol, were identified in the mixture. Catechol and quinol have both been identified previously in tobacco smoke[3] and the former has been reported as a constituent of tobacco leaf.[8] Isoeugenol, which was identified in the extract but was not found previously in the steam distillate, is characterized by two peaks when chromatographed, presumably owing to the *cis* and *trans* forms. This phenol is also a known constituent of tobacco[7] and tobacco smoke.[9]

EXPERIMENTAL

Preparation of Extract

1·5 kg of Latakia tobacco leaf was extracted according to a previously described method,[1] but with omission of the steam distillation. The crude phenolic extract in alkali was treated with excess Ac_2O and the acetates partitioned into ether. After evaporation of the solvent, the crude acetates were carefully distilled at a pressure of 25 Torr and the fraction boiling up to 210° collected.

Preparation of Authentic Compounds

A sample of 5-methylguaiacol, b.p. 118°/25 mm, was prepared by the reduction of isovanillin with Zn amalgam and HCl.[10] The reduction of syringaldehyde[11] with Zn amalgam and HCl yielded 4-methyl-2,6-dimethoxyphenol, b.p. 210–212°/25 mm. Similarly, 4-ethyl-2,6-dimethoxyphenol, b.p. 172°/25 mm, was prepared by the reduction of acetosyringone. The methylation of phloroglucinol with saturated methanolic HCl yielded the monomethyl ether, m.p. 69–71°, and dimethyl ether, m.p. 34–36°.[12]

The acetates were prepared by shaking an alkaline solution of the pure phenol with an excess Ac_2O. After allowing to stand for 5 min the acetates were extracted into ether, washed three times with $NaHCO_3$ solution, dried, ether evaporated and distilled.

In order to confirm the presence of 2,6-xylenol and *o*-ethylphenol, which were inseparable as their acetates, the anisole derivatives were prepared by dissolving a portion of the extract in 3 N NaOH solution and heating with dimethyl sulphate for 2 hr on a steam bath. The derivatives were extracted into ether, dried and evaporated to low bulk. The mixture was then vacuum distilled at 25 mm and the fraction with a b.p. of 80–100° collected.

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Gas Chromatography

All investigations were carried out on a Perkin–Elmer F11 gas chromatograph fitted with a flame ionization detector.

The stationary phases and conditions used were as follows:

(*a*) A support-coated open-tubular capillary column (15 m × 0·5 mm i.d.) coated with diethylene glycol succinate polyester (DEGS). N_2 carrier gas pressure: 3 p.s.i.g. Oven temperature: programmed 130–160° at 1·5°/min.

(*b*) A surface-coated stainless-steel open-tubular column, (50 m × 0·25 mm i.d.) coated with polyphenyl ether OS124 (PPE). N_2 carrier gas pressure 20 p.s.i.g. Oven temperature: 150–180° at 1·5°/min.

(*c*) A surface-coated stainless-steel open-tubular column, (50 m × 0·25 mm i.d.) coated with trixylenyl phosphate (TXP). N_2 carrier gas pressure: 20 p.s.i.g. Oven temperature: 150–180° at 1·5°/min.

(*d*) A surface-coated stainless-steel open-tubular column, (50 m × 0·25 mm i.d.) coated with Apiezon L. Carrier gas pressure: 20 p.s.i.g. Oven temperature: 150°.

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