THE QUALITATIVE COMPOSITION OF PEAT SMOKE

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Peat smoke, produced and collected in an apparatus which is described, was distilled to obtain compounds with boiling points which would not preclude their presence in malt whisky. An oil phase and an aqueous phase were obtained and these were analysed separately using GLC, TLC and a combination of GLC and Mass Spectrometry. A number of phenols, two furan aldehydes and series of alkanes, alkenes and aromatic hydrocarbons were found.

Key words: *analysis, gas chromatography, thin-layer, spectrometry, aldehyde, hydrocarbon, phenol, spirit.*

Introduction

In the traditional process of making malt whisky, the malt is given a prolonged kilning over a smouldering peat fire. The more modern oil-fired kilning of such malts has incorporated the introduction of peat smoke into the air stream passing through the malt. From tasting the malt, the wort or the final whisky, it is apparent that peat smoke makes a contribution to flavour. Furthermore it has been found that with the modern, rapid kilning it is more difficult to achieve as high a level of this flavour as was given by the traditional method.

Attempts to improve the efficiency and reproducibility of the peating process have relied on the measurement of phenols in the malt[2,4] because they are easily measured by colorimetric methods and they are most unlikely to arise in the subsequent processes of mashing, fermentation and distillation. However, it has been recognized that the smoke may contribute flavour substances other than phenols.

It was decided to carry out a comprehensive analysis of peat smoke as the first stage in a more detailed investigation of the problem of flavour contributions of peated malt to whisky.

Experimental

Elemental Composition of Peat.—A sample of peat was analysed using a C–H–N analyser and found to have the following composition (dry basis):

Hydrogen 6·4%; Carbon 57·5%; Nitrogen 0·79%.

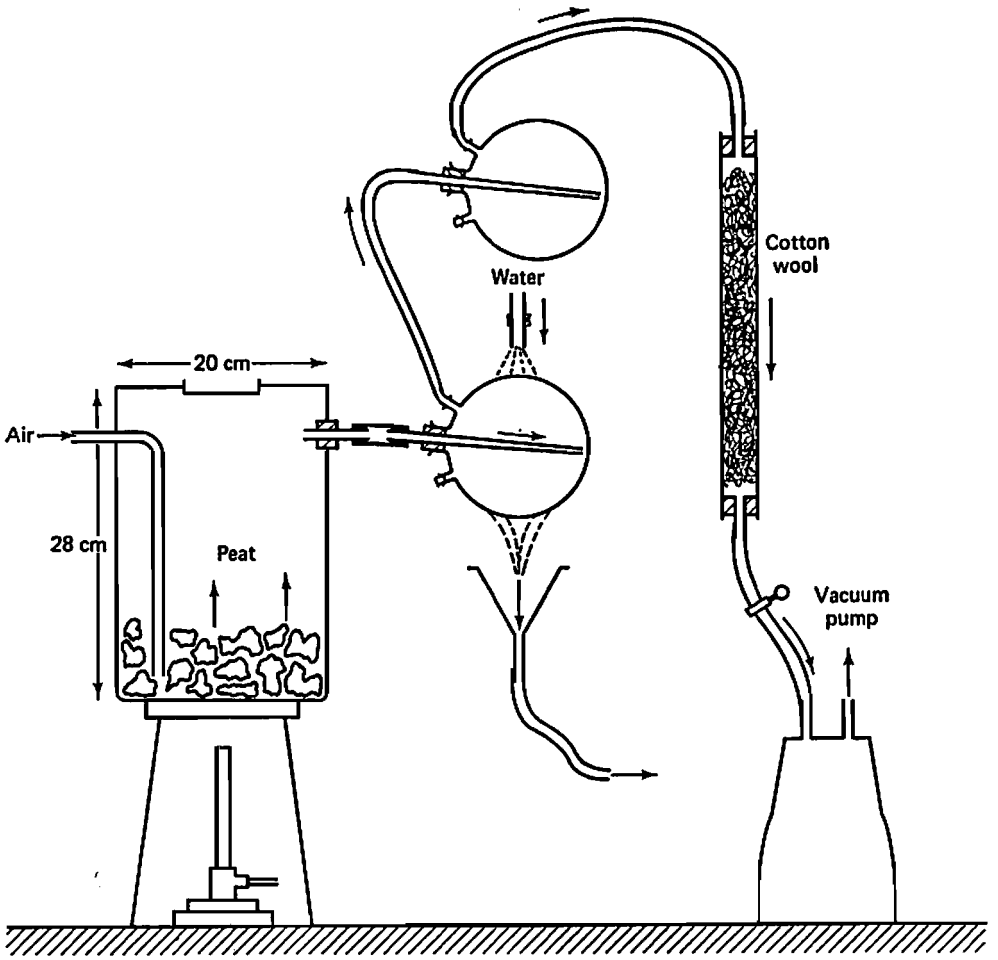

Fig. 1.—Peat-smoke generator/collector. The peat-container is a tin-plate can. The flasks are 1-litre, Quickfit, with a cooling water spray directed over the lower one. The glass jets entering the flasks are 1 mm in diameter.

Peat smoke oil (produced as described later) was subjected to sodium fusion, using the Lassaigne method. The solution was examined using the methods of organic qualitative analysis for nitrogen, halogens and sulphur. All were found to be absent, and it appeared therefore that peat smoke contained only compounds of carbon, hydrogen and oxygen.

Preparation of peat smoke concentrate.—Approximately 1 kg of peat was burned in the apparatus shown in Fig. 1. Air was drawn through the apparatus at a rate which just prevented loss of smoke from the inlet to the tin. The method of condensing smoke by passing it through a jet and allowing it to impinge on a cooled glass surface[6] proved very efficient and the cotton wool trap merely served to prevent the minor amount remaining from reaching the laboratory atmosphere.

The condensate was a mixture of an oil, a wax and an aqueous phase, and because the whisky process itself involves distillation, it was considered permissible to remove the oil and aqueous phase by distillation at atmospheric pressure.

The oil and the aqueous phase were separated and the latter was extracted twice with ether. The ether extracts were com-

bined and evaporated at 30° C in a vacuum oven. The oil and the extract of the aqueous phase were analysed separately.

Analysis of the Aqueous Phase

Gas chromatographic analysis was carried out using a Perkin–Elmer F 11 instrument fitted with two columns containing 10% Carbowax 20 M on Chromosorb W, previously treated with a silylating agent to reduce tailing. Nitrogen was the carrier gas and a flame ionization detector was employed. Unless otherwise stated, temperature programming between 100° C and 200° C at 5° C per min, was used.

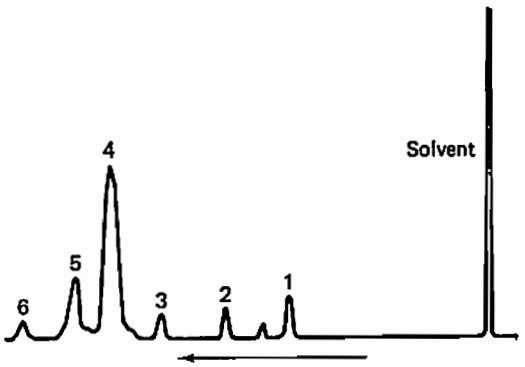

Fig. 2.—G.L.C. of aqueous extract. The peaks are: 1 = furfural; 2 = 5-methyl furfural; 3 = guaiacol; 4 = phenol; 5 = p-cresol; 6 = 3:5 xylenol.

GLC of the aqueous phase extract (Fig. 2) showed two peaks which were removed by treatment prior to chromatography by shaking with 2,4-dinitrophenylhydrazine in dilute sulphuric acid. By adding furfural and 5-methyl furfural to the extract, these peaks were enhanced and thus the presence of these aldehydes was strongly indicated.

5-Methyl furfural was not readily obtainable and was prepared from sucrose by the method of Rinkes.[5] The purity was checked by gas chromatography and the identity established by NMR and IR spectroscopy.

Isothermal GLC at 200° C indicated the presence of guaiacol, phenol, p-cresol, 2,3-xylenol and 3,5-xylenol peaks (Fig. 3) but the column was not capable of resolving a number of minor phenols from the main peaks.

A thin-layer plate with silica gel plus 13% calcium sulphate was prepared, spotted with

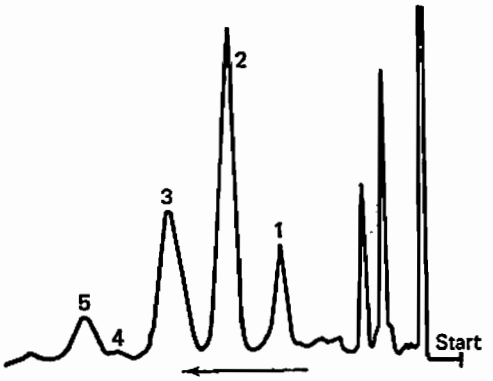

Fig. 3.—Isothermal G.L.C. of peat-smoke phenols. The peaks identified are: 1 = guaiacol; 2 = phenol; 3 = p-cresol; 4 = 2:3 xylenol; 5 = 3:5 xylenol.

the aqueous extract and the various phenols as marker substances and developed in chloroform. The spots were made visual with iodine vapour (Fig. 4).

In order to cross-check, 30 × 5 μl portions of the extract were spotted on to another 30-cm plate, 1 mm thick, together with composite marker spots at the sides. After development, the outer zones of the plate were developed and inner zones corresponding

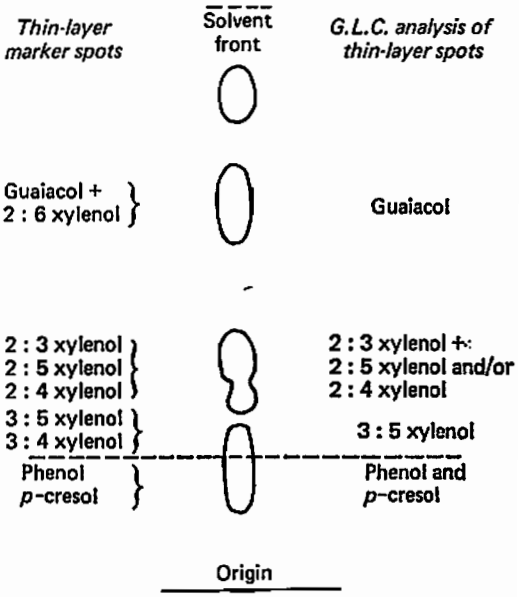

Fig. 4. Combined T.L.C./G.L.C. of peat-smoke phenols.

to the coloured areas were removed from the plate. These were separately extracted with ether, allowed to concentrate by evaporation and analysed by GLC. Fig. 4 shows the results obtained.

From the combination of GLC and TLC it appeared that phenol, p-cresol, guaiacol and 3,5-xylenol were major components, that small amounts of 2,4- and/or 2,5-xylenol may have been present and that both 3,4-xylenol and 2,6-xylenol were absent.

Using a more polar developing solvent for TLC, the following R_f values were found:

Phenol 0·49; m-Cresol 0·56; p-Cresol 0·56; o-Cresol 0·75.

It was thus possible to separate o-cresol from phenol by TLC although they were eluted together in GLC. Using a combination of TLC followed by GLC of the separate zones, the presence of o-cresol in the extract was demonstrated.

A number of alternative GLC columns were tried and it was found that 10% polyethylene glycol adipate on acid-washed Diatomite S gave the best separation. Temperature programming the column from 150° C to 200° C at the very slow rate of 1° C per min confirmed the presence of o-cresol, which appeared as a shoulder immediately after phenol. This column, however, did not separate p- and m-cresols.

Analysis of the Oil

The oil was analysed by GLC on a column of 10% Carbowax 20 M on silylated Chromosorb W, 80 to 100 mesh, using nitrogen as the carrier gas and a flame-ionization detector. Temperature programming from 100° C rising by 5° C per min to 200° C was employed. The instrument was a Perkin–Elmer F 11. Over thirty peaks appeared on the chromatogram.

In order to classify the compounds present according to their reactive groups, the oil was subjected to a number of reactions and re-chromatographed after each reaction. This provided evidence that any peak removed or greatly reduced by the treatment possessed the particular group involved. The reactions used have been summarized by Leathard & Shurlock[3] but these authors' reactions were carried out with comparatively volatile substances, and gas-phase reactions in a syringe were used. With the higher-boiling components found in peat smoke, the conditions of the various reactions were altered as follows:

Concentrated sulphuric acid.—1 ml of the oil was mixed with 1 ml of conc. sulphuric acid. Both components were previously cooled in a freezer. The mixture was shaken for 1 h and extracted with 5 ml of iso-pentane. The extract was allowed to concentrate by evaporation.

70% *Sulphuric acid.*—The above procedure was repeated using 70% sulphuric acid in place of the concentrated acid.

Sodium metal.—2 ml of the oil was dissolved in 20 ml of toluene and a 2-ml sample was withdrawn for comparative GLC.

3 g of sodium metal in thin slices was added to the remaining solution and the mixture was gently refluxed for 6 h. The solution was poured off, centrifuged, filtered through Watman No. 1 paper and chromatographed.

2,4-Dinitrophenylhydrazine (DNPH).—0·5 ml of the oil was dissolved in 5 ml of iso-pentane and shaken for 1 h with 10 ml of a solution containing 0·1 g of 2,3-DNPH and 2 ml conc. H_2SO_4 in 13 ml of water. The organic layer was separated and chromatographed.

Preliminary experiments had shown that when the reagent was shaken with iso-pentane the 2,4-DNPH stayed in the aqueous layer. When the reagent was shaken with either benzaldehyde or acetophenone, the derivative was removed from the organic layer.

Bromine.—2 ml of the solution of the oil in toluene which had been refluxed with sodium were shaken for 1 min with 20 ml saturated bromine water. The organic layer was separated, dried with sodium sulphate and chromatographed.

Hydroxylamine hydrochloride.—0·5 ml of the oil were dissolved in 5 ml di-iso-propyl ether and 1 ml was withdrawn for comparative GLC.

The remaining solution was shaken for 2½ h with 10 ml of an 8% solution of $NH_2OH.HCl$ in water. The organic layer was separated, dried with sodium sulphate and chromatographed.

Results of Preliminary Analysis

Sodium removes all classes of organics apart from ethers and hydrocarbons. From the oil it removed the four major phenols,

phenol, p-cresol, guaiacol and 3,5-xylenol and the two aldehydes, furfural and 5-methyl furfural, all of which had been found in the aqueous extract and which were present in smaller quantities in the oil. No other peaks were removed, suggesting that alcohols and ketones were absent.

Hydroxylamine hydrochloride will remove aldehydes, ketones and alcohols. Only the two aldehydes were removed; this is further evidence that alcohols and ketones were absent.

2,4-Dinitrophenyl hydrazine merely removed the two aldehydes, indicating that other carbonyl compounds, including ketones, were absent.

Concentrated sulphuric acid removes all classes except alkanes and aromatic hydrocarbons. Temperature-programmed chromatography of the extract showed a series of eleven prominent peaks; subsequent isothermal chromatography at 150° C gave a series of peaks where $\log_{10}$ (retention time) gave a straight-line relationship with carbon number (Fig. 5).

Similar chromatography of the material treated with 70% sulphuric acid showed a series of additional peaks when compared with the material treated with concentrated sulphuric acid. From Leathard & Shurlock's Table[3] these must be due to alkenes and isothermal chromatography again gave a straight-line relationship (Fig. 5). It appeared, therefore, that the oil fraction contained an homologous series of alkanes (or aromatic hydrocarbons) and an additional series of alkenes.

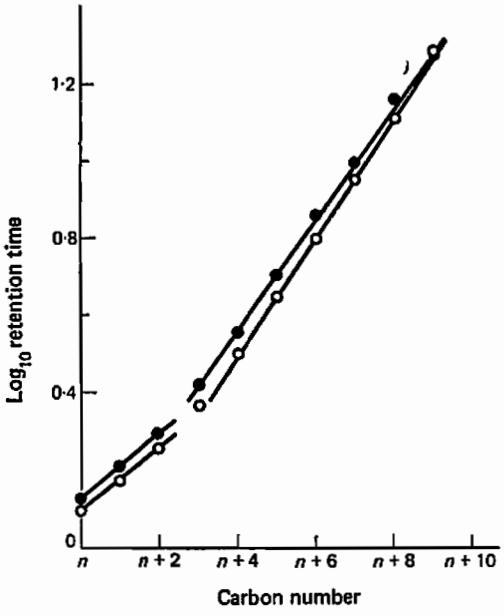

Fig. 5.—Isothermal G.L.C. at 150° C of alkane and alkene series in peat-smoke oil. Solid circles—alkenes. Hollow circles—alkanes.

The treatment with bromine-water gave additional evidence of the presence of alkenes. Ten peaks were removed by bromination and of these eight were found to be peaks stable to 70% sulphuric acid but not to concentrated sulphuric acid.

At this stage of the work, therefore, it appeared that peat smoke contained a number of monohydric phenols, two furan aldehydes, a series of alkanes (the lowest member being octane) and a series of alkenes (starting with octene), with possibly a number of aromatic hydrocarbons.

Gas Chromatography—Mass Spectrometry

The relative complexity of this mixture and the anticipated fairly low concentrations of some components meant that only two means of confirmation seemed feasible. Preparative gas chromatography followed by classical identification methods would probably have resulted in inadequate separation, producing fractions containing a number of isomers, for example of each alkene. Such fractions would give a mixture of di-bromo derivatives and have an "average" refractive index, thus the final identification would be rendered very difficult.

The alternative was to use a gas chromatograph coupled to a mass-spectrometer. It was accepted that this would not separate isomers, but the existence of, for example, several octenes, would not invalidate the confirmation of the presence of octene, although the technique would not establish the position of the double bond.

The gas chromatograph used was a Hewlett–Packard model 5750 with the effluent from the single column split in the ratio 1:1:1. One outlet fed a flame ionization detector, one fed a thermal conductivity detector (not recorded) and the third was connected with a Biemann fritted glass separator to an A.E.I. M.S.20 mass spectrometer, which is a low-resolution instrument employing magnetic scanning. The resolution was 1 in 475 at 10% valley.

Mass spectra were recorded at 70 eV at a scan speed of 10 s per decade. Spectra were recorded on a UV Recorder with sensitivities of 1x, 10x and 100x. A spectrum was recorded each time a component eluted from the chromatograph entered the ion chamber, *i.e.*, when the total ion current meter reading reached a maximum.

Helium at a flow rate of 30 ml/min was used as the carrier gas and the column was temperature programmed from 40° C to 200° C at a rate of 2° C/min.

For the separation and identification of furfural and 5-methyl-furfural in the aqueous fraction a Porapak column gave sufficient resolution. However, to obtain sufficient resolution of components in the oil fraction, Carbowax 20 M (polyethylene glycol) on Chromosorb W, 80 to 100 mesh had to be used.

The columns were made from stainless-steel tubing 2 m long × $\frac{1}{8}$ in external diameter.

Notes on the Interpretation of the Mass Spectra

(1) Reference spectra were taken from Cornu & Massot.[1]

(2) Corrections for the background spectra were not made, because peaks were eluted too close together to enable the background to be measured, except at the beginning and end of the run. However, these backgrounds showed prominent peaks at m/e 41, 43, 55, 56, 57, 69, 70, 71, 83 and 85. It follows that these peaks are frequently enhanced in the spectra found, when compared with reference spectra.

(3) Where a chromatographic peak consisted of more than one substance the minor constituent (*e.g.* toluene in peak 5) was identified by its molecular ion (*e.g.* 92) and the presence of any characteristic fragment (*e.g.* 91). The other fragments quoted in the literature were often markedly enhanced, because they also occurred in the major component.

(4) With unedecene and alkenes higher in the series, the base peak (defined by the reference spectrum) was less than some of the other peaks found in the experimental run. This effect arose from the presence of minor amounts of the corresponding alkane (demonstrated by the presence of its molecular ion), the enhanced peaks being common to the spectra of both hydrocarbons.

(5) Spectra were corrected to two significant figures because of the effects outlined above and because, when two sets of spectra were quoted in the Tables, they frequently did not show sufficient agreement to justify the accuracy implied by three figures.

Sequence of Identification.—The results obtained by chemical modification were substantiated by the mass-spectra. Further confirmation was obtained by adding each compound found, in turn, to the original oil (or aqueous phase) and re-chromatographing. A few compounds (marked with an asterisk in Table II) were not added to the original oil for confirmation, because they were not readily obtainable.

TABLE I

MASS SPECTRA OF SELECTED COMPONENTS OF PEAT SMOKE

$M = \frac{m}{e}$; R = Relative abundance from Reference Tables; F = Relative abundance found

From the aqueous extract										
Furfuraldehyde M.W.96										
M	96	95	39	38	29	37	97	67	40	42
R	100	91	40	11	11	6	6	6	4	4
F	100	100	100	29	43	18	19	23	13	15
5-Methyl furfuraldehyde M.W.110										
M	110	109	53	27	29	28	51	39	50	43
R	100	89	77	48	26	24	22	21	19	19
F	100	100	83	57	19	27	18	26	13	30
From the oil										
Peak(1) Octane M.W.114										
M	43	41	29	57	85	27	56	42	39	70
*R	100	38	35	34	30	29	18	16	14	12
F	100	41	29	53	24	17	28	21	12	17
Peak (2) Octene M.W.112										
M	43	41	55	56	42	70	29	27	36	69
**R	100	91	76	66	64	61	58	57	46	31
F	100	59	54	41	26	28	35	22	15	28
Peak (22) Pentadecane M.W. 212										
M	57	71	43	41	85	55	56	42	70	39
†R	100	57	49	48	35	21	15	13	10	7
F	100	56	104	60	32	51	37	17	27	27
5-Methyl furfural										
M	110	109	53	27	29	28	51	39	50	43
R	100	89	77	48	26	24	22	21	19	19
F	100	89	42	52	54	135	35	38	12	152
Peak (35) Phenol M.W.94										
M	94	66	39	65	40	95	38	55	63	50
R	100	28	28	22	13	11	10	8	6	6
F	100	23	49	28	23	19	12	54	14	12
p-Cresol M.W.108										
M	107	108	77	51	79	39	53	50	52	78
R	100	93	24	19	19	17	15	14	9	8
F	100	128	67	56	44	117	44	28	22	22

* n-Octane ** 1-Octene † n-Pentadecane

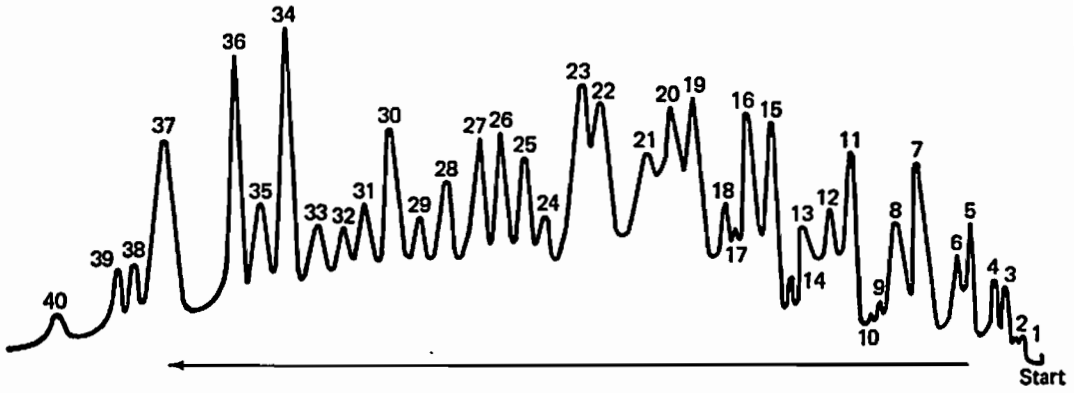

Fig. 6.—Flame ionization detector trace during G.L.C./M.S. of peat-smoke oil. A key to the numbers is given in Table II.

Results of Gas Chromatography—Mass Spectrometry

For reasons of brevity, only a representative number of mass-spectra found are quoted (Table I).

The GC–MS analysis of the aqueous extract confirmed the presence of furfural and 5-methyl furfural in peat smoke. The results for peat smoke oil are summarized in the G.C. Chart (Fig. 6) and in Table II.

The analysis of the phenols described earlier can only be confirmed in general terms by mass-spectrometry because of the similar spectra given by the three cresols and by the various xylenols.

TABLE II

Compounds Present in Peat-Smoke Oil
(The numbers refer to Fig. 6)

1	Octane
2	Octene
3	Nonane
4	Nonene
5	Decane + toluene
6	Decene
7	Unedecane + C_8 aromatic hydrocarbon
8	Unedecene
9	C_9 aromatic hydrocarbon + unedecene
10	C_9 aromatic hydrocarbon
11	Dodecane
12	Dodecene
13	C_{10} aromatic hydrocarbon
14	C_{10} aromatic hydrocarbon (probably methyl-n-propyl benzene with a base peak of $\frac{m}{e}$ 105)
15	Tridecane
16	Tridecene
17	Tridecene
18	C_{11} aromatic hydrocarbon
19	Tetradecane + furfuraldehyde
20	Tetradecane + furfuraldehyde
21	Tetradecene + tetradecane + C_{12} aromatic hydrocarbon
22	Pentadecane + 5-methyl-furfuraldehyde
23	Pentadecane
24	C_{12} aromatic hydrocarbon + pentadecane + pentadecene
25	Pentadecene + C_{12} aromatic hydrocarbon + pentadecene
26	Unknown + pentadecane + pentadecene
27	Naphthalene + hexadecane*
28	Hexadecene* + C_{14} aromatic hydrocarbon*
29	Propyl propenyl benzene* + hexadecane* + C_{14} aromatic hydrocarbon*
30	Propyl propenyl benzene* + hexadecane* + C_{14} aromatic hydrocarbon*
31	Methyl naphthalene* + guaiacol + hexadecane*
32	Methyl naphthalene* + guaiacol + hexedecane*
33	Small quantities of the above three compounds, indicated by the presence of their molecular ions
34	Heptadecane*
35	Phenol + cresol + heptadecane*
36	Phenol + cresol + traces of octadecane* and octadecene*
37	Cresol + xylenol
38	Cresol + xylenol
39	Cresol + xylenol
40	Cresol + xylenol

Note—In "mixed peaks" the one giving the most abundant mass spectrum is listed first.

* Substances not readily obtainable which, therefore, have not been added to the oil for further confirmation of their presence by G.L.C.

Summary of Qualitative Composition.—The compounds which occur in peat smoke and which could, because of their volatility, have an influence on the flavour of malt whisky have been shown to comprise a number of monohydric phenols, two furan aldehydes and three series of hydrocarbons, alkanes, alkenes and aromatic hydrocarbons, together with naphthalene and methyl naphthalene.

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Note Added in Proof

After this paper was submitted the use of a GLC column specifically developed for phenols by Brooks* has been investigated. The stationary phase was 5% tris-2,4-xylenyl phosphate on acid-washed Celite (100 to 120 mesh).

This column gives a better separation of phenols than those described in the paper. Its use in analysing peat smoke phenols has demonstrated the presence of both 2,4- and 2,5-xylenol together with traces of 2,3-xylenol. Separation of phenol and p-cresol was quite distinct and m-cresol appeared as a definite "shoulder" immediately after p-cresol.

In our analyses the 3,5-xylenol coincided with m-ethyl phenol and p-ethyl phenol peaks. Therefore, in the work described above, the peak described as 3,5-xylenol may consist of one or more of these three substances.

Work is continuing to confirm the identification of this peak and to quantify all the phenols as percentages of the total phenol fraction.

* Brooks, V. T., *Chemistry & Industry*, 1959, 1317.