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FURTHER ASPECTS OF THE CHEMISTRY OF CIGARETTE SMOKE—II*

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Abstract—By vapour phase chromatography it has been shown that the acids in cigarette smoke simpler than lauric contain both even and odd numbers of carbon atoms, and the C_8, C_9 and C_{11} unsaturated acids also appear to be present. A new weak base has been separated from the hydrochloric acid extract of the smoke. From the neutral fraction (M_N of Paper 1) a number of compounds have been isolated by chromatography one of which is low melting and appears to be $C_{29}H_{48}O$

In Part I, Table 1 records the results obtained in a preliminary examination of the smoke from eight different brands of cigarettes and Table 2 the analysis of the methyl esters of the simpler acids in the very complex acid mixture in the smoke. Of the total of these simpler acids 30 per cent contain at least 15 acids simpler than lauric, with saturated and unsaturated members present to about the same extent. In view of the possible important role the acids might play,[1] this 30 per cent fraction has been further analysed, through the kindness of Dr. R. V. Crawford† and shown to be more complex than was suggested earlier.

The bases in cigarette smoke. In addition to those already recorded the hydrochloric acid extract obtained after acetic acid has removed the more strongly basic compounds contains a significant amount of a base whose I.R. absorption spectrum suggests the presence of a lactam group, and which does not appear to have been mentioned previously. A curious point found in working up this material is that successive treatments with hydrochloric acid eliminated small amounts of a neutral material whose properties and analysis agreed with hentriacontane (H.T.C.) although this does not form a complex with nicotine itself.

M_N. This fraction (Part I, Table 1) contains the neutral compounds including the hydrocarbons and the nitrogenous materials not removed by hydrochloric acid, or potassium in an inert solvent but are removed by lithium aluminium hydride. Although M_N contains a *very small* proportion of aromatic hydrocarbons much work has been done in recent years on the isolation and identification of any member with known carcinogenic action and especially of 3:4-benzpyrene. From spectrographic evidence this compound appears to be present to the extent of about 1 p.p.m.[2] and its presence appears to have been confirmed since Wynder and Hoffman state that two crystals of the compound were obtained by the sublimation of the product from 200 paper chromatograms.‡ The M_N fraction as a whole appears to be markedly terpenoid including steroid in character and only a slight separation can be effected

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* Part I: G. R. Clemo, *Tetrahedron* 3, 168 (1958).
† Research Dept., J. Bibby and Sons Ltd.
‡ *London Symposium* July (1959).

[1] *J. Roy. Soc. Health* 79, 74 (1959).
[2] H. R. Bentley and J. G. Burgan, *Analyst* 83, 442 (1958).

by distillation in a molecular still at 10 ^{4} mm. A more complete separation results from chromatography on alumina giving products whose analyses indicate a $C_{15}H_{24}$ compound with two double bonds, which is thus bicyclic and a $C_{20}H_{32}$ compound. Neither of these combines with maleic anhydride or has so far given a crystalline bromide. An interesting constituent is a low melting compound indicated by analysis to be $C_{29}H_{46}O$ with 5 double bonds, (although $C_{30}H_{48}O$ is not ruled out). It is, therefore, probably monocyclic. Rather surprisingly it gives no test for unsaturation with tetranitromethane but gives a light brown colour in acetic anhydride and sulphuric acid which changes after about 3 hours to a dull purple suggestive of a sterol. It is unchanged by refluxing with acetic anhydride alone (or in pyridine) and gives no oxime. Its I.R. absorption spectrum shows no evidence for carbonyl or conjugation, but for a hydroxyl group of uncertain type. This compound comes off the alumina column just before the sterols of which β- and γ-sitosterol and stigmasterol have been recorded as present recently by other workers.

There is some uncertainty in the literature about the melting points, specific rotations and solvent of crystallization of both β- and γ-sitosterol and whilst some workers give 137° as the m.p. of the β-compound, others state that it forms crystals with ½ H_2O with a m.p. of 137·5°. The information on γ-sitosterol appears even less definite, but it appears to melt at 147–148°, although plates with 1 H_2O are also stated to melt at 147–148°. According to Heilbron *et al.*[3] two fully reduced stigmasterols, exist one of m.p. 127°, giving an acetate of m.p. 123°. From the sterol fractions coming off the column it is easy to obtain highly crystalline specimens melting at 137° and 147–148° which would appear to be β- and γ-sitosterol respectively, and whilst the I.R. absorption spectrum of the substance melting at 137° agrees substantially with that of β-sitosterol both spectra show peaks at 970 cm ^{1} which are characteristic of the C 22–23 side chain double bond of stigmasterol. This would also explain the low specific rotations found for the 137° and 147–148° materials e.g. $[\alpha]_D$, ·46° for the 137° melting material (literature value of 37°). It is stated[4] that the side chain double bond of stigmasteryl acetate is selectively reduced over palladized charcoal in ethyl acetate to give β-sitosterol acetate and it was hoped to be able thus to eliminate the stigmasterol from the mixture coming off the column and thereby obtain pure β- and γ-sitosterols. When this experiment was kindly carried out by Dr. F. J. McQuillin the analysis of the resulting product indicated a reduction to stigmastanol and the I.R. absorption spectrum showed differences from that of β-sitosterol, but no material is available for a direct comparison. Some of the compounds described above were first recorded in an interim report to a committee of the Medical Research Council in 1956.

Although the M_X fraction from all the brands of cigarettes mentioned in Table 1 (*loc. cit.*) have not been examined in detail it can be stated that all the compounds described above have been obtained from the U.S., Russian, German, and French cigarettes referred to. Further, in view of the great increase in the use of tipped cigarettes it was of interest to examine whether the smoke from such tipped cigarettes differed substantially from the smoke issuing when the tips were removed. Although only two brands of popular tipped cigarettes have been so far examined it has been found that the tips effect approximately a 7 per cent reduction in the amount of M_X

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[3] Heilbron, Jones, Roberts and Wilkinson, *J. Chem. Soc.* 344 (1941).

[4] Bernstein and Wallis, *J. Org. Chem.* 2, 341 (1938).

(per 100 g of tobacco) and that all the neutral compounds described above can be isolated from the smoke of the tipped cigarettes. It is of course possible that the tips selectively remove some injurious material not so far identified.

EXPERIMENTAL

All carbon hydrogen analyses and M.Ws are by Drs. Weiler and Strauss (Oxford) the oxygen determinations and most of the double bond estimations have been kindly carried out by Miss J. Cuckney (Imperial College) the rotations by Dr. F. J. McQuillin.

Methyl esters. The ether soluble free acid and phenolic mixture (15 g) was steam distilled giving 2·33 g of product and leaving non-volatile materials—including all hydroxy and di-carboxylic acids, of which 11·6 g was ether soluble and 0·36 g ether insoluble (evidence for the presence of unstable compounds). The 2·33 g in ether was thoroughly extracted with sodium carbonate solution and thus separated into phenols (1·61 g) and acids (0·51 g). The latter were esterified (MeOH + H_2SO_4) and on working up the product 0·22 g of esters boiled between 45°–80°/2 mm (n^{20} = 1·4878). This 0·22 g was reduced in methanol with PtO_2/H_2 (6 cc absorbed in the first hour and 7 cc altogether) giving a pale yellow oil, b.p. 90–140°/20 mm n^{20} = 1·4846. This simplified ester mixture has been shown by an efficient Reoplex vapour-phase column to contain at least 25 different esters. The amounts present from even numbered carbon atoms is rather small (nC_{14}, 2%; nC_{12}, 2%; nC_{10}, 5%; nC_8, 5%). A comparison of the curves given by the ester mixture before and after reduction shows an increase in the C_8, C_9 and C_{11} acid ester content after reduction, thus pointing to the unreduced mixture containing unsaturated C_8, C_9 and C_{11} components.

The HCl extracted basic mixture. When 1·45 g of this mixture was steam distilled 0·27 g of a yellow sweet smelling oil passed over. After treatment with sodium nitrite the material was again steam distilled, dissolved in hydrochloric acid, and freed from traces of non-basic material by extraction twice with ether –a yellow mobile oil (0·15 g b.p. 85°/2 mm) being obtained. (Found: C, 79·1, 78·8; H, 7·55, 7·6; N, 9·2, 9·3, 8·4. $C_{19}H_{22}ON_2$ requires: C, 77·5; H, 7·5; N, 9·5; $C_{20}H_{22}ON_2$, C, 78·8; H, 7·2; N, 9·1%). No reliable M.W. values or a crystalline picrate or picrolonate have been obtained. I.R. absorption bands 1700, 1450 and 750 cm^{-1} suggest the presence of $>N \cdot CO$, $>NMe$ and phenyl groups. The above non-basic material gave soft prisms from benzene–alcohol, m.p. 65° not depressed by admixture with H.T.C. (Found; C, 85·7; H, 13·9; Calc. for $C_{31}H_{64}$: C, 85·3; H, 14·7%).

M_x. When the neutral fraction is steam distilled 6–7% passes over as a mobile camphoraceous oil (b.p. 75°/2 mm M.W. about 170). It gives the $C(NO_2)_4$ test for unsaturation and catalytic reduction indicates the presence of one double bond but so far no crystalline derivative has been obtained. The steam non-volatile material, dissolved in light petroleum (10 cc, b.p. 60–80°), deposits on standing an amorphous solid (0·035 g) from which no pure substance has been obtained. The light petroleum soluble material (2·07 g) in a molecular still (10^{-4} mm) gave 0·3 g up to 100°, 0·15 g 100–150° and 0·68 g 150–250°, leaving 0·89 g of residue. From the first fraction materials corresponding to *a* and *b* below can be obtained but nothing definite from the remainder or residue.

Separation on alumina. Four separate lots of 1 g of the light petroleum soluble material in light petroleum (10 cc) were chromatographed on 20 × 0·8 cm columns of alumina, giving *a* (0·5 g, not adsorbed). Five elutions with light petroleum (10 cc) gave *b* (1·4 g) from the first washing and *c* (0·5 g) from the second to fifth (orange coloured) washings. Five elutions with light petroleum (10 cc) containing 10% benzene gave *d* (0·255 g), five elutions with 50% benzene–light petroleum mixture e_1 (0·1 g) from the first, and e_2 (0·3 g) from the second to fifth elutions. Five further elutions with benzene (10 cc) gave *f* (0·28 g), five with benzene containing 10% ether *g* (0·29 g), five with ether, *h* (0·35 g), five with benzene containing 10% ethanol *i* (0·1 g), and finally ethanol containing 10% acetic acid eluted *j* (0·08 g, benzene soluble). All weights after evaporation included the weight of traces of porous pot.

Fraction a. When dissolved in a small volume of acetone a trace of H.T.C. separated and by chromatographing the soluble material in light petroleum on alumina a colourless peppery smelling oil was obtained which nearly all distilled at 80°/2 mm approx (Found: C, 87·8; 87·7; H, 11·8; 12·1. M, 199. $C_{15}H_{24}$ requires: C, 88·2; H, 11·8; M. 204). The oil showed marked unsaturation with tetranitromethane and catalytic reduction indicated the presence of two double bonds. (Found: C, 86·4; H, 13·0. $C_{15}H_{28}$ requires: C, 86·5; H, 13·5%).

Fraction b. When treated as for *a* gave a thick oil, b.p. 130°/2 mm approx (Found: C, 87·8, 87·8; H, 11·9, 11·7; M, 278, 286: $C_{20}H_{32}$ requires: C, 88·2; H, 11·8%; M, 272.).

Fractions c. On standing some months in winter this solidified and when extracted with methanol gave 0·17 g of nearly white prisms, m.p.25–27°, raised to 27° by one further crystallization. The melt after solidification usually had m.p. 35°–36°. (Found: C, 84·8; H, 11·2; O, 3·63; M, 402, 400; $C_{29}H_{46}O$ requires: C, 84·9; H, 11·2; O, 3·9%; M, 410 $C_{30}H_{48}O$ requires: C, 84·9; H, 11·3; O, 3·8%, M, 423). The compound had only a small, if any specific rotation $[\alpha]_D$ −1·2° and in a catalytic reduction 9·299 mg absorbed 2·593 ml H_2 at N.T.P. which on the basis of $C_{29}H_{46}O$ indicated five double bonds.

Fractions d and e. These were chromatographed over alumina and only more of the above compound was obtained.

Fraction e_2. This material in light petroleum was adsorbed on a 10 × 0·8 cm alumina column and washed successively with 10 lots of light petroleum (10 cc), 5 lots of light petroleum containing 10% benzene, and in 5 lots of benzene-light petroleum mixture when all the material was recovered. The first material eluted gave a trace of a soft crystalline solid but that from the 2nd to 15th washings only gums. A brown band was eluted in the 16th and 17th washings (0·13 g) which gave colourless prisms m.p. 130° from methanol.

Fraction f. This 0·28 g crystallized easily from light petroleum, so it was dissolved in light petroleum containing 10% benzene and then chromatographed as under e_2. In this case 10% ether in benzene and finally 10% ethanol in benzene were needed to elute the last trace of material, but nothing definite could be isolated from these eluates. The last runnings with the 10% benzene gave 15 mg, m.p. 128–130° once crystallized from methanol, whilst the 50% benzene runnings gave fractions of 0·06 g, 0·025 g and 0·05 g all of which were sparingly soluble in, and crystallized well from, methanol giving products: m.p.132–136°, 135°–137° and 145°–148° respectively. All the above crystalline fractions gave the usual sterol colour reactions and on recrystallization the 135–137° material melted at 137° and the 145–148° gave striking prisms m.p.148° (Found for the 137° material: C, 83·94, H, 12·1; and for the 148° material C, 83·5; H, 11·7. $C_{29}H_{50}O$ requires: C, 84·0; H, 12·1%).

Acetylation and reduction. 0·1 g of the combined crystalline fractions when refluxed with acetic anhydride gave 0·09 g of colourless plates, m.p.125°. When 83·3 mg were reduced in ethyl acetate (10 cc) with palladized charcoal/hydrogen, 4·6 cc hydrogen were absorbed at 20°, corresponding to 1·05 moles. H_2 per mole. The reduced product crystallized from methanol in prisms, m.p.125° and after hydrolysis with methanolic potassium hydroxide 37 mg of prisms were obtained from acetone, m.p.127°. (Found: C, 82·0; H, 12·7; $C_{29}H_{52}O$ requires: C, 83·6; H, 12·5%).

Fractions *g*, *h*, *i* and *j* have so far given only indefinite results.

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