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SOME CONSTITUENTS OF CITY SMOKE

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Abstract—In view of the complementary action between the neutral constituents of city smoke and cigarette smoke in producing cancer in mice, the former has been further investigated and a number of aromatic hydrocarbons isolated and identified.

THE preliminary observations recorded on Newcastle-on-Tyne city smoke[1] were followed by the separation and identification of many constituents of cigarette smoke.[2] When it was found that atmospheric smoke and cigarette smoke exert a complementary action in cancer production in mice,[3] it was decided to isolate and identify the aromatic hydrocarbons in the atmosphere. The neutral portion of atmospheric soot extracted with light petroleum was nitrogen free. Chromatography of this complex mixture yielded a number of hydrocarbons from which the crystalline, 1,3,5-trinitrobenzene complexes were prepared (the latter usually crystallize better than the picrates). It was found experimentally that aromatic hydrocarbons are eluted from alumina in the following order: naphthalene, fluorene, anthracene, phenanthrene, pyrene, 1,2- and 3,4-benzpyrenes, chrysene, 1,12-benzperylene and picene. A few hydrocarbons have been separated chromatographically on highly acetylated cellulose powder[4] and thanks to the kindness of Professor W. Cocker this technique has been applied successfully to the separation of 1,2- and 3,4-benzpyrenes.

The separation of hydrocarbons from their complexes could be achieved chromatographically on a long column of alumina, the hydrocarbon coming off first, or by warming a benzene solution of the complex with Zn dust and HCl. The complex form chrysene is split by solution in EtOH but most such complexes crystallize well form EtOH. The mixture of hydrocarbons obtained required further chromatography over alumina, vacuum sublimation and crystallization in order to effect their separation.

Thus, whilst the UV spectra of crystalline solids from some of the eluates m.p. 225–245° show that chrysene is the major component, it is difficult to isolate pure chrysene from it except through the 1,3,5-trinitrobenzene complexes. Although pure chrysene is readily obtained on chromatographing a mixture with phenanthrene, anthracene and pyrene, a crystalline specimen, m.p. 230–232° (eluate 10) was shown by MS to contain chrysene and three homologues with M.Ws 242, 256 and 270 in the ratio of 1:0·7:0·4:0·4. Analysis of a 1,3,5-trinitrobenzene complex from this mixture indicates a methylchrysene.

In the picene fraction m.p. 305–310°, the major component was picene as shown by m.p. IR and UV spectra. The MS indicated the presence of an additional five

[1] Presidential address, G. R. Clemo, Section B, Brit. Ass. Advance. Sci. (1953).
[2] G. R. Clemo, *Tetrahedron* **3**, 168 (1958); *Ibid.* **11**, 11 (1960).
[3] G. R. Clemo and E. Miller, *Brit. J. Cancer* **14**, 65 (1960).
[4] Th. Wieland, G. Lüben and H. Determann, *Experientia* **18**, 430 (1962).

compounds with M.Ws 252, 266, 292, 306 and 320 in the ratio of picene 1:0·07:0·4: 0·4:0·15:0·04. Although picene does not appear to form a picrate or a 1,3,5-trinitrobenzene complex it forms a crystalline derivative with 2,4,7-trinitrofluorenone which could not be isolated in a pure form from the above mixture. A minor complication is that colourless hydrocarbons are often coloured by a trace of adhering impurity, e.g. tetracene, imparting a yellow colour to chrysene. Further, the C and H combustion values of many different hydrocarbons are almost identical and many isomeric alkyl derivatives of hydrocarbons do not appear to have been characterized by UV and IR spectra and, in any case, the UV spectra may not distinguish between some of the alkyl derivatives as, for example, 3',4', or 5'-methyl-3-4-benzphenanthrenes 1,2-Benzpyrene can be easily isolated from some eluates from the neutral portion whereas the 3,4-isomer could not be isolated from them in a pure form although its presence was proved by chromatography using acetylated paper. An equal mixture of these isomers gave 1,3,5-trinitrobenzene complexes which could not be resolved by crystallization.

It is of interest that whilst 3,4-benzpyrene was used in 823 painting experiments on mice between 1932 and 1947 only one such experiment which showed some activity was done with 1,2-benzpyrene.[5]

So far pyrene, 3-methylpyrene, 1,2-benzpyrene, triphenylene, chrysene, 1,12-benzperylene and picene (impure) have been isolated and identified and 3,4-benzpyrene identified but the non-aromatic hydrocarbon constituents forming the bulk of the neutral portion have yet to be identified.

Basic fraction. The basic fraction of the light petroleum extract is a complex mixture of which one fifth is slowly volatile in steam and M.S. of the most volatile part of the distillate shows it to contain a series of compounds with M.Ws 179, 185, 193, 199, 207, 211, 213, 221, 225, 227 and 230 from which a base analysing for $C_{13}H_{15}N$ has been isolated.

EXPERIMENTAL

The chromatographic experiments have been carried out using alumina (Brockmann) in tubes of 15 mm i.d. (T) or 7·5 mm i.d. (T_1) Silica gel did not give more helpful results.

The sublimations have been done in tubes of 5 mm i.d. which after evacuation to 2 mm and sealing are 12 cm long. These tubes are heated in a narrow metal tube containing 10 cm of iron filings so that with the bottom of the glass tube 1 cm from the bottom of the filings a temperature of 235–245° is maintained. When the sublimate is clearly in zones as with chrysene, pyrene and phenanthrene, they are termed 'a', 'b' and 'c' from the bottom. When anthracene is substituted for phenanthrene only 'a' and 'b' are obtained, 'a' giving chrysene and 'b' a mixture of the other two hydrocarbons m.p. 120–150°.

Collection and extraction of atmospheric soot. The atmospheric soot was collected on cotton wool filters. In a battery of 54 filters air was drawn during 10–12 weeks. The cotton wool from one such filter with the adhering soot was extracted with light petroleum (b.p. 60–80°) in a Soxhlet for 6 hr when on an average 10·1 g of tar 'A' was obtained. A further extraction with benzene removed 4 g of pitch 'B' which was not investigated.

Extraction of a clean cotton filter. Clean cotton wool from one unused filter was similarly extracted with light petroleum (b.p. 60–80°) for 6 hr when 1·4 g of a buttery solid was obtained. This was dissolved in a small volume of light petroleum and 0·48 g of a colourless solid separated on cooling m.p. 78–80°, on crystallization 82°. (Found: C, 81·9, H, 14·1. Calc. for octacosanol-1 $C_{28}H_{58}O$: C, 82·0; H, 14·1%.) It gives an acetyl deriv m.p. 68°.

The material in the light petroleum filtrate was shaken with 1N KOH leaving 0·61 g neutral

[5] *Survey of Compounds tested for Carcinogenic Activity* (2nd Edition) National Cancer Institute, Bethesda, Md. (1951).

material from which acids extracted *no* bases. When this was chromatographed over alumina (T_1) and eluted with light petroleum (5 ml b.p. 60–80°) the first 5 eluates gave a mixture of paraffins and eluates 20–22 gave a small amount of a compd which on crystallization gave colourless plates, m.p. 71°. (Found: C, 81·05; H, 13·2. Calc. for eicosanol-1, $C_{20}H_{42}O$ (m.p. 71°) C, 80·5; H, 14·1%.)

The alkaline extract was wholly acidic and vacuum sublimation and crystallization from 95% MeOH gave 0·3 g palmitic, m.p. 61·2° and stearic acid m.p. 70°.

Separation of aromatic hydrocarbons by alumina

Exp. I. A soln of 10 mg of each of naphthalene, fluorene, anthracene, phenanthrene, pyrene, 1,2- and 3,4-benzpyrenes and chrysene in light petroleum was dropped down a 20 cm column of Al_2O_3(T) and eluted with light petroleum. On working up the eluates by crystallization or by sublimation and crystallization it was found that the hydrocarbons came down in the above order except that no appreciable separation of 1,2- and 3,4-benzpyrenes was effected.

Exp. II. A soln of 5 mg of each of chrysene, 1,12-benzperylene and picene in warm benzene was dropped down a 20 cm column of $Al_2O_3(T_1)$ and eluted with warm benzene. On working up the eluates as above it was found that the hydrocarbons came down in the order, chrysene, 1,12-benzperylene and picene.

Separation of tar 'A'. Tar 'A' (30·3 g) from three smoke filters was warmed with ether (400 ml), 0·37 g being left undissolved. The soln was shaken thrice with IN KOH (50, 40 and 25 ml) when 22·4 g was recovered from the ether and 6·8 g of acids and phenols from the alkaline extract. The 22·4 g of material was dissolved in benzene (50 ml) and shaken thrice with 20% AcOH (40, 25 and 25 ml) when 21·74 g was recovered from the benzene extract and 0·32 g bases from the acid extract.

The 21·74 g material (only a trace being steam volatile) was extracted with a total of 80 ml light petroleum when 4·1 g residue remained. 25 ml extract was run down each of 4 tubes T containing 20 cm of alumina in darkness and washed successively with 20 ml light petroleum (×10), 20 ml of 10% benzene–light petroleum (×10), 20 ml benzene (×5) 20 ml of 10% EtOH–benzene (×5) and 20 ml EtOH (×5). The alumina was still brownish yellow with dark bands at the top but a final extraction with EtOH in a Soxhlet gave only a small amount of tar. The first solvent down gave 0·82 g of material and eluates 1–35* a total of 15·77 g material made up of 2·8, 1·05, 0·72, 0·49, 0·54 (quickly crystallized) 0·32, 0·2, 0·16, 0·2, 0·16, 0·11, 0·27, 0·22, 0·16, 0·12, 0·15, 0·11, 0·11, 0·06, 0·11, 0·04, 1·36, 0·5, 0·26, 0·25, 0·2, 2·8, 1·85, 0·17, 0·08 and the last five EtOH eluates 0·15 g.

The 3·62 g from the first down and eluate 1 (about 10% coming from the cotton filters) was a mixture of unbranched aliphatic compds with M.Ws 436, 450, 464, 478, 492 and 506 and probably lower members. By distillation and crystallization hentriacontane (436) was obtained, m.p. 68°. (Found: C, 85·2, H, 14·4. Calc. for $C_{31}H_{64}$: C, 85·3, H, 14·7%.)

Eluate 2 (1·05 g) was also a mixture of paraffins and *eluates* 3 *and* 4 gave faintly yellow solns in light petroleum and set to a paste from which eicosanol-1, m.p. 71° was obtained. The filtrate from this was run down a 20 cm column of alumina (T_1) and eluted with light petroleum. The third eluate gave a red soln of 1,3,5-trinitrobenzene complex but no solid was obtained (nor with picric acid or 2,4,7-trinitrofluorenone), whilst eluates 4–28 gave considerable amounts of 1,3,5-trinitrobenzene complexes. The m.p. of the various fractions after crystallization varied from 215–216° (eluate 4), 222–224° (eluates 6 and 7) and 218–222° (eluates 11–28). Regeneration of the hydrocarbon mixture either by dropping the light petroleum–benzene soln of the complex down $Al_2O_3(T_1)$, or by warming its benzene soln with Zn dust and HCl gave a crystalline product m.p. 78–105°. On sublimation the middle zones of 'a' and 'b' melted at 84° and 135° respectively. Crystallization of the former from MeOH gave plates, m.p. 95–110° and from the filtrate by crystallizing from 95% MeOH colourless plates were obtained m.p. 73–74° not depressed by authentic 3-methylpyrene. This gave a 1,3,5-trinitrobenzene complex, m.p. 220° (that for 3-methylpyrene has m.p. 222–223°). (Found: C, 64·0; H, 3·7; N, 9·35. Calc. for $C_{23}H_{15}O_6N_3$: C, 64·3; H, 3·5; N, 9·8%) and a picrate m.p. 202°. (Found: C, 62·3; H, 3·4; N, 9·4. Calc. for $C_{23}H_{15}O_7N_3$: C, 62·0; H, 3·4; N, 9·4%.) IR: 3·26, 6·28, 6·98, 7·6, 8·05, 8·45, 10·38, 11·9, 13·35 and 14·1 μ.

Sublimation and crystallization of the 135° m.p. solid gave prismatic plates of pyrene. UV (0·001% soln in EtOH 335, 321, 307, 287, 273, 263, 241 and 233 mμ. IR 3·255, 6, 6·26, 6·98, 7·62, 8·05, 8·44, 11·9, 13·38 and 14 mμ. The 95–110° m.p. solid depressed the m.p. of 3,4-dimethylpyrene (m.p. 103–104°) but no 3,5-dimethylpyrene (m.p. 98–99°) was available for comparison and it is probably a mixture of pyrene and 3-methylpyrene.

Eluate 5 gave 25 mg eicosanol-1, m.p. 71° from soln in light petroleum and the filtrate gave 1,3,5-trinitrobenzene complexes of the mixture of pyrenes reported above.

Eluate 6 gave a similar result. On standing a soln of *eluate* 7 in light petroleum (1 ml) 15 mg of solid separated including some highly crystalline warts of impure chrysene. The filtrate gave 20 mg of 1,3,5-trinitrobenzene complexes as amber prisms, m.p. 198–200° after crystallization. Extraction of the 15 mg solid with light petroleum gave eicosanol-1, but the remaining material and the hydrocarbons regenerated from the complexes have not been identified.

Eluate 8 gave 16 mg of colourless solid from light petroleum (1 ml) m.p. 160–195° and the filtrate 12 mg of 1,3,5-trinitrobenzene complexes (after crystallization) m.p. 193° after softening from 183°. Sublimation of the 16 mg solids (in two tubes) and extraction of the 'a' zones with light petroleum (b.p. 40–60°) left 6 mg which, on crystallization from light petroleum, gave 2 mg of colourless plates, m.p. 240–250° and which gave a 1,3,5-trinitrobenzene complex, m.p. 186–187° identical with that from chrysene. UV (0.005% soln of the m.p. 240–250° material in EtOH 362, 344, 320, 307, 295, 269, 244, 223 mμ in close agreement with the values for chrysene.

From the 'b' zones and the filtrate from 'a' by running the light petroleum soln down alumina, eluate 6 gave 7 mg crystalline material m.p. 222°, from which a 1,3,5-trinitrobenzene complex was obtained as amber prisms from EtOH (twice) m.p. 168°. (Found: C, 65·6; H, 4·0. $C_{25}H_{17}O_6N_3$ requires: C, 65·9; H, 3·7%.) 4-Methylchrysene has m.p. 224–225°.

The 12 mg of 1,3,5-trinitrobenzene complex and 9 mg of similar material from eluate 9 (below) in 20% benzene–light petroleum was dropped down a 20 cm column of alumina (T_1) and 9 mg material obtained from eluates 2, 3 and 4, which when crystallized from light petroleum gave faintly yellow needles. When these were sublimed and zone 'a' crystallized the long colourless needles characteristic of triphenylene were obtained, m.p. 194–195°. UV (EtOH) 302, 285, 274, 259, 250 λ_{max}.

Eluate 9 gave 11 mg material from soln in light petroleum m.p. from 175–200° and the filtrate 9 mg of 1,3,5-trinitrobenzene complex m.p. 193° after crystallization. Sublimation of the 11 mg and crystallization of zone 'a' gave 5 mg of material m.p. 230–235°.

Eluate 10 gave 6 mg of slightly yellow solid (m.p. 175–190°) from light petroleum and the filtrate 18 mg of 1,3,5-trinitrobenzene complex, after recrystallization m.p. 228–230°. Sublimation of the 6 mg material and crystallization of zone 'a' gave faintly yellow plates m.p. 228–232°. This and the 5 mg material from eluate 9 above were recrystallized to give colourless plates m.p. 230–232°. UV (0.001% soln in EtOH 320, 306, 293, 284, 269, 260, 242 and 223 mμ in agreement with the values for chrysene. MS showed that it contains 4 compds of M.W. 228 (chrysene) 242, 256 and 270 (possibly chrysene homologues) in the approximate ratio of 1.0:0·70:0·40:0·04. The 18 mg of the 1,3,5-trinitrobenzene complex in 50% benzene–light petroleum was dropped down a 20 cm alumina column (T_1) and eluted with 10% benzene light petroleum. Eluates 2, 3 and 4 gave 9 mg of yellow solid which on sublimation gave yellow prisms, m.p. 155° and 168° from zones 'a' and 'b' respectively. Resublimation of the former and recrystallization of the sublimate with the latter gave well formed yellow plates, m.p. 176–177° unchanged by admixture with 1,2-benzpyrene but depressed to 130–150° by 3,4-benzpyrene. UV (0·002%) soln in EtOH 368, 360, 350, 332, 318, 307, 291, 280, 264, 261, 240, 225 mμ in close agreement with the values for 1,2-benzpyrene. In addition, two peaks at 437 and 411 resemble the two long wave peaks of perylene which would account for the yellow colour of the 1,2-benzpyrene. The yellow prisms, m.p. 155° were chromatographed (by Professor Cocker) along with authentic samples of 1,2- and 3,4-benzpyrene on acetylated paper using as eluting mixture EtOH–toluene–water (17:4:1). The chromatogram showed a component whose R_F value was the same as that of 1,2-benzpyrene and another whose R_F value and fluorescence colour was the same as that of 3,4-benzpyrene.

Eluates 11–13 all gave gums from soln in light petroleum and the filtrates gave 40 mg of 1,3,5-trinitrobenzene complex m.p. 195–220° raised to 224° on recrystallization, from which 1,2-benzpyrene was obtained as above.

Eluates 14 *and* 15 gave a trace of amorphous solid from light petroleum and the filtrate 31 mg of 1,3,5-trinitrobenzene complexes which gave reddish-brown prisms, m.p. 202–204° after recrystallization from which orange plates, m.p. 225° were obtained (alumina). The UV and IR spectra suggest that it is a deriv of pyrene but this has not been confirmed.

Eluates 16 *and* 17 gave only a trace of solid from soln in light petroleum and the filtrate gave 35 mg of 1,3,5-trinitrobenzene complexes, m.p. 190–220°, raised to 233° by recrystallization. Its soln in 50% benzene–light petroleum was run down a 20 cm alumina column (T_1) and washed with

10% benzene–light petroleum when eluates 1–4 gave 7 mg of solid which sublimed almost completely as zone 'a' and gave an intense violet fluorescent soln in light petroleum and crystallized from it as reddish-yellow plates m.p. 268° depressed to 240° by perylene but not by 1,12-benzperylene (m.p. 282°). MS shows that the material contains compounds of M.W. 276, 252, 282, 290 and 300 in the ratio of 1:0·029:0·011:0·006 and 0·004. UV (EtOH) 385, 363, 347, 330, 301, 289, 277, 254, 224, 212 mμ. The first five values agree with those for 1,12-benzperylene and this is confirmed by IR 3·28, 6·1, 6·62, 6·9, 7·18, 7·45, 7·6, 8·32, 8·72, 11·84, 12·3, 13·02, 13·28, 14·79 and 15·4 mμ. Thus, the 268° m.p. material is nearly pure 1,12-benzperylene and the MS peaks at 290 and 300 could be due to a methyl deriv and coronene respectively.

Eluates 18–21 gave 7 mg solid from light petroleum, m.p. 260° and the filtrate 47 mg 1,3,5-trinitrobenzene complexes m.p. 232° with softening from 200°. The 7 mg solid sublimed, to give a highly crystalline zone 'a', m.p. 273–275°, which gave the lemon-yellow plates m.p. 283–285° on recrystallization. It depressed the m.p. of 1,12-benzperylene to 225–230° and of 3,4,9,10-dibenzopyrene (m.p. 283–284°—kindly supplied by Dr. E. Clar) to 250° and has not yet been identified. Recrystallization of the 47 mg complexes from EtOH gave amber prisms m.p. 225–232°. When 15 mg complexes in benzene–light petroleum were run down an alumina column, eluates 2 and 3 gave 8 mg material which sublimed as red prisms, softening from 225° and melting finally at 242°. MS shows that it contains no less than 7 compounds with M.Ws 276, 282, 290, 298, 304, 306 and 318 in a ratio of 450:7:140:20:38:10:9. Since picene does not give a 1,3,5-trinitrobenzene complex it cannot account for the major component.

Eluate 22 which was port wine coloured, gave 1·36 g material all of which was soluble in light petroleum (12 ml). After standing 2 hr the soln deposited 28 mg of a yellow, tacky solid. This gave yellow plates from 10% benzene–light petroleum, m.p. 298–305° raised to 305–310° by a second crystallization. Sublimation and recrystallization raised the m.p. to 325°, the material melting on the slide, whereas picene (m.p. 364°) sublimed by 325° without melting. Picene gives a 2,4,7-trinitrofluorenone deriv as thin light red needles m.p. 245–247°. MS shows that the m.p. 305–310° material contains compds of M.W. 278, 252, 266, 292, 306 and 320 in the approximate ratio of 1:0·07:0·4:0·4:0·15:0·04. That the m.p. 325° material is mainly picene is shown by UV (sat soln in EtOH) 274, 264, 256, 251 and 230 mμ. These values agree with those for picene and this is confirmed by IR 3·3, 6·2, 6·91, 7, 7·78, 7·92, 8·85, 9·78, 10·6, 11·36, 11·58, 12·38, 12·96, 13·24, 13·53, 13·76, 14·23 and 14·89 mμ. Small peaks at 12·8 and 12·9 mμ. are not due to picene.

Eluate 23 gave a small amount of high m.p. solid similar to that from eluate 22, whilst *eluates* 24–26 gave only tars from soln in light petroleum and the filtrates gave small amounts of 1,3,5-trinitrobenzene complexes as brownish red powders, m.p. 180–210°. The material from *eluates* 27–28 gave a reddish-brown soln in EtOH from which only octacosanol-1, m.p. 82° separated. The addition of 1,3,5-trinitrobenzene to the filtrate gave no ppt and shows the virtual absence of aromatic hydrocarbons.

Basic fraction. About one-fifth was slowly volatile in steam and alumina separates the volatile bases into 'a', 'b', 'c' and 'd' fractions. Fraction 'a' gave a low energy MS showing the presence of compounds with M.Ws 179, 185, 193, 199, 207, 211, 213, 221, 225, 227 and 230 and IR indicates the possible presence of the NH group. Distillation of the most volatile part of 'a' gave a sweet smelling base. (Found: N 7·4, 7·7. $C_{13}H_{11}N$ requires: N 7·6%.) The picrates from the various fractions do not crystallize satisfactorily.

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