498. *The Formation of Aromatic Hydrocarbons at High Temperatures. Part I. Introduction.*

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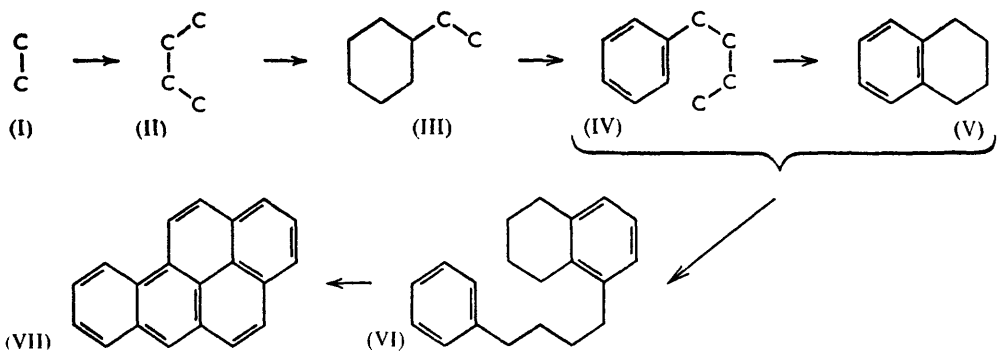
A mechanism for the formation of 3 : 4-benzopyrene from simple components is considered. The techniques used to separate and identify components of complex hydrocarbon tars are reported.

When 3 : 4-benzopyrene was first isolated from coal tar [1] it was recognised as the probable cause of certain "industrial" cancers. In recent years, however, 3 : 4-benzopyrene and other polycyclic aromatic hydrocarbons have been identified in a great variety of materials; [2–12] this widespread occurrence has obvious implications in connection with the incidence of cancer among the general population, and it is important to determine the origin and mode of the formation of such carcinogenic hydrocarbons.

The carcinogenic hydrocarbons which occur in human environment seem to be formed only at high temperature. The "primary" tar produced by heating coal at 300—450° in absence of air consists mainly of paraffins, *cyclo*paraffins, olefins, and phenols. At higher temperatures this mixture undergoes various transformations to give a "secondary" tar containing a greater proportion of polycyclic aromatic compounds. Kennaway [13] found that although tar produced from a Durham Holmside coal at 450°



had slight carcinogenic activity, tars produced at 560° and at 1250° were much more potent. Similarly, certain petroleums became carcinogenic after being heated,[13] and Scottish shale oil was carcinogenic only after pyrolysis.[14] Carcinogenic tars and tars containing polycyclic aromatic hydrocarbons have been produced by pyrolysis of

[1] Cook, Hewett, and Hieger, *J.*, 1933, 395.
[2] Goulden and Tipler, *Brit. J. Cancer*, 1949, **3**, 157; Passey, *Brit. Med. J.*, 1922, ii, 1112.
[3] Sulman and Sulman, *Cancer Res.*, 1946, **6**, 366; Beránková and Sůla, *Časopis Lékařů, Českýeh*, 1953, **92**, 195; *Chem. Abs.*, 1955, **49**, 592.
[4] Cooper, *Chem. and Ind.*, 1953, 1364; Cooper and Lindsey, *ibid.*, 1953, 1177; 1260; Clemo, *ibid.*, 1953, 557; 1955, 38; Waller, *Brit. J. Cancer*, 1952, **6**, 8; Leiter, Shimkin, and Shear, *J. Nat. Cancer Inst.*, 1942, **3**, 155; Shore and Katz, *Analyt. Chem.*, 1956, **28**, 1399.
[5] Falk, Steiner, Goldfein, Breslow, and Hykes, *Cancer Res.*, 1951, **11**, 318.
[6] Falk and Steiner, *ibid.*, 1952, **12**, 30; von Haam and Mallette, *Arch. Industr. Hyg.*, 1952, **6**, 237.
[7] Kotin, *Cancer Res.*, 1956, **16**, 375.
[8] Gilbert and Lindsey, *Chem. and Ind.*, 1956, 927.
[9] Hougen, *ibid.*, 1954, 192.
[10] Campbell and Lindsey, *Chem. and Ind.*, 1955, 64; 1957, 951.
[11] *Idem, Brit. J. Cancer*, 1956, **10**, 649.
[12] Cooper, Lindsey, and Waller, *Chem. and Ind.*, 1953, 1205; 1954, 1418; Gilbert and Lindsey, *Brit. J. Cancer*, 1956, **10**, 642; Bonnet and Neukomm, *Helv. Chim. Acta*, 1956, **39**, 1724; Patton and Touey, *Analyt. Chem.*, 1956, **28**, 1685; Philippe and Hobbs, *ibid.*, 1956, **28**, 2002; Gilbert and Lindsey, *Brit. J. Cancer*, 1956, **10**, 646.
[13] Kennaway, *Brit. Med. J.*, 1925, ii, 1.
[14] Berenblum and Schoental, *Brit. J. exp. Path.*, 1943, **24**, 232.

cholesterol,[15] skin,[13] yeast,[13] isoprene,[13,16] acetylene,[13] diacetyl,[17] and aliphatic hydrocarbons from tobacco.[18]

The formation of aromatic hydrocarbons from aliphatic compounds at high temperatures has not been extensively studied. It seems, however, that the most important reactions are cracking, diene syntheses, dehydrogenations, and cyclodehydrogenations. Polycyclic compounds may be formed by a series of such reactions, and the present work was designed to study the formation of 3 : 4-benzopyrene (VII) and related carcinogenic hydrocarbons at high temperatures. As a working hypothesis it has been assumed that 3 : 4-benzopyrene may be formed by the series of reactions (I→VII). This presupposes that benzopyrene will be formed by pyrolysis of any of the intermediate compounds, and has governed the initial choice of materials to be pyrolysed. The state of hydrogenation of the intermediate compounds would seem to be unimportant as hydrogenations and dehydrogenations occur easily at high temperatures.

The available experimental evidence, which is meagre, supports the view that benzopyrene is formed from simpler units by a stepwise synthesis. Pyrolysis of acetylene (I) is known to give aromatic hydrocarbons,[19] and the crude tar is carcinogenic.[13] Its fluorescence spectrum is diffuse but not inconsistent with the presence of 3 : 4-benzopyrene.[20] In this connection, incomplete combustion of organic compounds often gives acetylene,[21] and acetylene has been detected in tobacco smoke.[22] A four-carbon unit (II), such as vinylacetylene or butadiene, seems the most logical intermediate in the formation of the " tars," and small quantities of the former have been reported among the products.[22] Butadiene undergoes diene synthesis with itself to give vinyl*cyclo*hexene, and the pyrolysis of butadiene yields relatively large amounts of ethylbenzene and styrene (III).[23] Naphthalene and tetralin (V) were also formed in this pyrolysis and the complex mixture of polycyclic compounds contained 3 : 4-benzopyrene.[23]

A clue to the nature of the final steps of the synthesis is provided by the observation that tetralin and aluminium chloride give a complex mixture which becomes carcinogenic after being heated.[24] This carcinogenic tar has a diffuse fluorescence spectrum in the same region as that of 3 : 4-benzopyrene. 6-4′-Phenylbutyltetralin is known to be a major constituent of the mixture, and it has been suggested[25] that some 5-4′-phenylbutyltetralin (VI) may also be formed and pass, rather easily, into 3 : 4-benzopyrene by cyclodehydrogenation.

Although this mechanism has been used as a working hypothesis, it is unlikely that any polycyclic compound will be formed by any single scheme. Moreover, the supposed intermediates may be produced from fragments larger than acetylene, and complex hydrocarbons need not necessarily break down to two-carbon fragments before re-synthesis. The C_6–C_4 fragments (IV and V), for example, could arise by cyclisation of a C_{10} hydrocarbon such as *n*-decane.[26] or from a higher hydrocarbon (such as dicetyl) by cracking followed by cyclisation.

The experimental section describes some general methods and techniques used in the investigations.

[15] Kennaway and Sampson, *J. Path. Bact.*, 1928, **31**, 609; Falk, Goldfein, and Steiner, *Cancer Res.*, 1949, **9**, 438.
[16] Kennaway, *J. Path. Bact.*, 1924, **27**, 233.
[17] Lam, *Acta Path. Microbiol. Scand.*, 1956, **39**, 198.
[18] *Idem*, *ibid.*, 1955, **37**, 421; 1956, **39**, 207.
[19] Berthelot, *Ann. Chim.*, 1866, **9**, 445; Zelinsky, *Compt. rend.*, 1923, **177**, 882; *Ber.*, 1924, **57**, 264.
[20] Kennaway, *Biochem. J.*, 1930, **24**, 497.
[21] Nieuwland and Vogt, " The Chemistry of Acetylene," Reinhold, New York, 1945.
[22] Fishel and Haskins, *Ind. Eng. Chem.*, 1949, **41**, 1374.
[23] Weizmann *et al.*, *ibid.*, 1951, **43**, 2312, 2318, 2322, 2325.
[24] Schroeter, *Ber.*, 1924, **57**, 1990; Kennaway, *Biochem. J.*, 1930, **24**, 497; Hieger, *ibid.*, 1930, **24**, 505.
[25] Cook and Hewett, *J.*, 1933, 398; cf. Grove, *J.*, 1953, 483.
[26] Rozenberg, *Doklady Akad. Nauk S.S.S.R.*, 1950, **73**, 719; see also, Hansch, *Chem. Rev.*, 1953, **53**, 353.

Experimental

Furnace.—This consisted of a silica tube (3 ft. × 1 in. i.d.) wound along its length with 25 s.w.g. Nichrome wire (total resistance 90 ohms). A calibrated chromel–alumel thermocouple was inserted through a small hole bored near the centre of the tube. The tube was mounted along the centre of a pressed asbestos-board box (3 × 1 × 1 ft.) filled with " vermiculite." The material to be pyrolysed was passed through a second silica tube (3 ft. 6 in. × ¾ in. i.d.) which just fitted into the heated tube. The furnace was inclined at 10° to the horizontal to facilitate collection of products.

Chromatography on Alumina.—Hexane or benzene was generally used as solvent and/or eluant. Thiophen-free benzene was washed with concentrated sulphuric acid and then water, and distilled. Hexane was also washed with concentrated sulphuric acid, then water, and fractionally distilled.

Gas–Liquid Partition Chromatography.—This was of great value in analyses of mixtures formed by pyrolysis of acetylene, styrene, etc. A Pyrex column (3 ft. × 6 mm. i.d.) was packed with alkali- and acid-washed Celite 545 (30—60 mesh) coated with Apiezon L high-vacuum grease (4 g./8 g. of Celite). The column and detecting and reference cells were mounted in a box (3 ft. × 1 ft. × 6 in.) of asbestos board, lined with aluminium foil. Heating was provided by three 1-kw radiator bars mounted in the box at one end, air being circulated by a powerful fan. Baffles increased circulation and prevented heating by radiation. The detecting and reference cells were A2321/100 thermistors,[27] sealed in T-tube Pyrex cells and enclosed in a brass " heat sink." The electrical detecting circuit was of normal bridge type [28] with the detecting and reference thermistors in the two arms of the bridge and 100-ohm fixed resistors in the other arms. A bridge voltage of 4—8 volts was used. Nitrogen was used as carrier gas, and the sample was injected by hypodermic syringe through a rubber cap. Solid samples were injected as solutions in xylene, mesitylene, or tetralin.

Each fraction was collected as it left the column. For the lower-boiling products a V-tube collector was connected *via* a B10 joint to the end of the detecting cell through a hole cut in the hot-air bath, the end of the V being cooled. For higher-boiling products a straight tube, with air cooling, was used. Polycyclic compounds were separated by chromatography under vacuum, a two-tube collector (two parallel tubes sealed to a B10 joint), with air cooling, being used. The dimensions of the collector were such that, when connected to the collector cell, the seal was well inside the hot-air bath. Under normal conditions the gas passed through one tube and only when a particular fraction was to be collected was the gas flow switched to the other; for this purpose a two-way tap was connected to the two tubes by pressure tubing.

Identification of Products.—Fractions from gas–liquid chromatograms were provisionally identified by comparison of retention times or of the ratio of their retention times to those of known substances under the same conditions. The identification was confirmed by collecting the fraction and determining the infrared, ultraviolet, or fluorescence spectrum or, in appropriate cases, by mixed m. p. determination and the preparation of derivatives. Fractions separated by fractional distillation and chromatography on alumina were similarly identified.

Infrared Spectra.—These were determined with a Grubb-Parsons double-beam instrument. Solids were examined as solutions in carbon tetrachloride. The spectra were compared with published curves, or with curves obtained from authentic specimens.

Fluorescence Spectra.—A direct-vision hand spectroscope (R. & J. Beck, Ltd.) calibrated from 400 to 700 mμ in 10 mμ divisions was used. A prism was fitted to cover half the slit so that unknown and standard solutions could be examined simultaneously. Provision was made for momentary illumination of the scale by a bezzle lamp, the light being diffused by frosted-glass plates. A 35-mm. camera, with a 50-mm. focal-length lens, and fitted with a 2 dioptre supplementary lens, was mounted directly in front of the eyepiece. The whole apparatus, except the camera, was enclosed in a light-tight box. The excitation radiation was from an enclosed 125 w mercury lamp, fitted to the outside of the box. Visible light was almost completely removed by passage through Wood's glass windows, leaving mainly the 3650 Å mercury line. The instrument was calibrated against mercury, neon, hydrogen, and sodium lines. In the 400 mμ region the expected accuracy is within 1 mμ, and in the 500 mμ region, within 5 mμ. Standard solutions were in the following concentrations: 3 : 4-benzopyrene, 5×10^{-4} mole/l.,

[27] Ambrose and Collerson, *J. Sci. Instr.*, 1955, **32**, 323.
[28] Dibat, Porter, and Stross, *Analyt. Chem.*, 1956, **28**, 290.

anthracene, 10^{-3} mole/l., and 1 : 2-benzanthracene, 5×10^{-4} mole/l. The solvent was usually benzene; but unknown products were always examined in the same solvent as the standard materials. With solutions of the above concentration a spectrometer slit-width of 0·01 in., and an exposure time of 1 min. at *f*2·9 with Kodak Tri-X film were usually satisfactory. The scale was illuminated for 0·2—0·5 sec. With this apparatus, 3 : 4-benzopyrene (5×10^{-4} mole/l. in benzene) gave a fluorescence spectrum showing considerable fine structure (Table 1).

TABLE 1. *Maxima* (mμ) *of fluorescence bands for* 3 : 4-*benzopyrene.*

Weigert [a] (acetone)	Chalmers [b] (alcohol)	Schoental [c] (light petroleum)	Cardon [d] (benzene)	Present work (benzene)
—	394	—	—	398 weak, B *
404	404·5	403	—	404 strong, N
410	410	408	410	409 weak, N
417	417	415	413	417 weak, N
427	429	427	—	427 strong, B
—	434	431	432	433 strong, B
—	—	437	—	437 v. weak, N
455	456	454	455	456 strong, B
485	—	—	—	485 weak, B

* B = Broad band; N = Narrow band.

[a] Weigert and Mottram, *Nature*, 1940, **145**, 895. [b] Chalmers, *Biochem. J.*, 1938, **32**, 271. Schoental and Scott, *J.*, 1949, 1683. [d] Cardon, Alvord, Rand, and Hitchcock, *Brit. J. Cancer*, 1956, **10**, 485.

TABLE 2. *Maxima* (mμ) *of fluorescence bands for perylene.*

Radulescu [a] (benzene)	Radulescu [a] (heptane)	Schoental [b] (light petroleum)	Present work (benzene)
446	440	438	445 B
475	465	465	475 B
505	498	497	502 B
537·5	535	—	536 B

[a] Radulescu and Dragulescu, *Bul. Soc. chim. România*, 1935, **17**, 9, 26, 35. [b] Schoental and Scott, *loc. cit.*

With weaker or impure solutions only the strong bands were normally detected. Perylene showed maxima as recorded in Table 2.

Ultraviolet Spectra.—Most of these were measured in 95% ethanol on a Hilger Uvispek spectrophotometer.

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