Colouring Matters of the Aphididæ. Part VIII. Studies on the Nature of the Aromatic Ring System in the Erythroaphins.*

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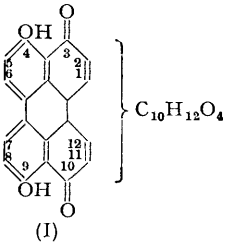
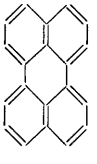
Fusion of erythroaphin-*sl* with zinc dust yields a complex mixture of hydrocarbons from which fractions have been separated showing the characteristic ultra-violet absorption of perylene, 1 : 12-benzoperylene, and coronene derivatives. The nature of the carbon skeleton of the erythroaphins is discussed in the light of this evidence, and that of the similarity between the absorption spectrum of perylene and of tetra-acetyldihydroerythroaphin.

Earlier papers in this series (*J.*, 1950, 477, 485, 3304; *Nature*, 1948, **162**, 759) have described the isolation of protoaphins, the characteristic pigments of the hæmolymph of many aphid species, and their stepwise conversion into the deep red erythroaphins. For reasons associated mainly with the availability of insect material, structural investigation of the latter has so far been largely confined to erythroaphin-*fb* derived from the black aphid *Aphis fabae* and erythroaphin-*sl* derived from the willow aphid *Tuberolachnus salignus*, although there is evidence that other members of the erythroaphin group exist (Part V, *J.*, 1951, 2633). Erythroaphin-*fb* and -*sl* are isomeric polycyclic dihydroxyquinones of formula $C_{30}H_{22}O_8$; they have identical ultra-violet absorption spectra and are remarkably similar in their general chemical behaviour. There can be no doubt that they contain the same chromophoric system and so they can be used to some extent indiscriminately in endeavouring to settle the nature of the aromatic nucleus of the erythroaphins; this is fortunate since the availability of individual aphid species, and hence of the two



(I)



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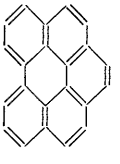

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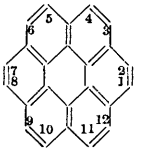


(IV)

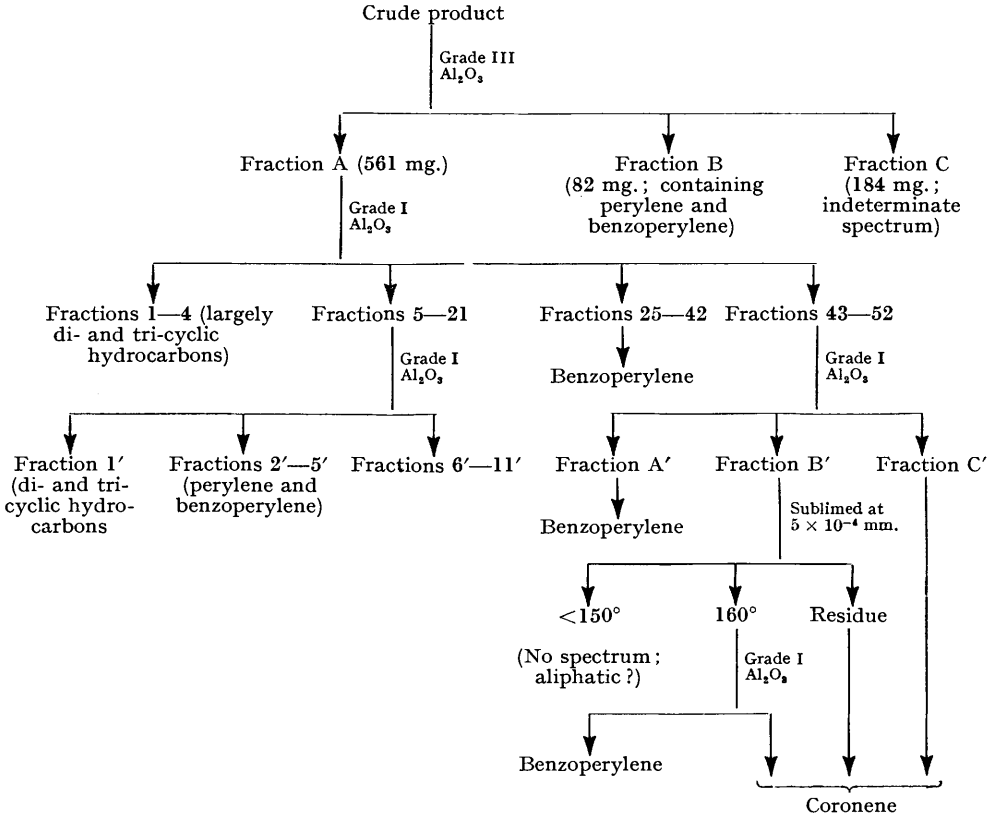
erythroaphins, varies considerably from year to year. Spectroscopic studies (Part V; Johnson, Quayle, Robinson, Sheppard, and Todd, *J.*, 1951, 2633) suggested that the quinone system in the erythroaphins extends through more than one ring. The observation that erythroaphin-*fb* (and, as will be shown in a later paper, also erythroaphin-*sl*) undergoes Thiele acetylation and reacts readily with ammonia and amines to give diamino-derivatives, or with alkaline potassium permanganate to give dihydroxyerythroaphin-*fb*, adds strong

* Part VII, *J.*, 1952, 4928.

support to this view (Part VII, *J.*, 1952, 4928). These reactions, which are interpreted as the normal addition reactions of quinones, indicate that the rings containing the quinone-carbonyl groups must contain unsubstituted positions, *i.e.*, that they cannot be in rings flanked on both sides by benzenoid nuclei as in anthraquinone. The fact that diamino-derivatives are produced under mild conditions supports the concept of an extended quinone system since 1 : 4-naphthaquinone under similar conditions yields only mono-amino-derivatives. The simplest expression covering these and other facts (*e.g.*, the formation of mellitic acid on oxidation of erythroaphins with nitric acid) was the partial structure (I) for the two erythroaphins (Part VII, *loc. cit.*).

Attempts to obtain definite evidence for the nature of the aromatic ring system present in the erythroaphins by oxidative degradation have so far yielded little information and other methods of attack have been examined. Of these, fusion with zinc dust has given the most decisive results. Since its introduction by Graebe and Liebermann (*Ber.*, 1868, **1**, 49), distillation with zinc dust has been widely used for the conversion of polycyclic hydroxyquinones into the parent hydrocarbons; although the method generally gives extremely low yields, the various aromatic hydrocarbons can be readily recognised, even in trace amounts, by their characteristic ultra-violet and visible absorption spectra (cf. Clar,

*Scheme for Chromatographic Separation of Hydrocarbons from Zinc Dust Fusion of Erythroaphin-*sl.



" Aromatische Kohlenwasserstoffe, Springer Verlag, Berlin, 1952). Fusion with zinc dust in a zinc chloride–sodium chloride melt (Clar, *Ber.*, 1939, **72**, 1645) is a practical improvement over the traditional distillation procedure, and in our experiments we applied it to erythroaphin-*sl.*

After many preliminary experiments the optimum procedure for the zinc dust fusion

was established as a preliminary fusion at 215—220° for 10 minutes, followed by further heating at 290° for 15 minutes. Even under these conditions the overall conversion into hydrocarbons was very low and the reaction was doubtless complicated by pyrolytic decomposition of the erythroaphin (Part V, *loc. cit.*). Moreover the yield of mixed hydrocarbons decreased on increasing the scale of individual fusions (cf. Witkop, *Annalen*, 1943, **554**, 122) so that it was necessary to do each experiment with only 20 mg. of erythroaphin-*sl*; in all 250 such experiments were carried out. The combined products from all these fusions were freed from inorganic material and phenols, and then carefully fractionated by chromatography on aluminium oxide followed, in the case of some fractions, by sublimation. The course of the separation is summarised in the Table. The nature of the products was established by their ultra-violet absorption spectra, determinations being made on every fraction throughout the separation process.

As a result of this work three aromatic ring-systems were detected, *viz.*, perylene (II), 1 : 12-benzoperylene (III), and coronene (IV). As far as we are aware this is the first occasion on which any of these systems has been found in the degradation products of a

FIG. 1. *Absorption spectra of benzoperylene* (——) *and the benzoperylene fraction* (. . . . .) *from the zinc dust fusion of erythroaphin*-sl (*in* 95% EtOH).

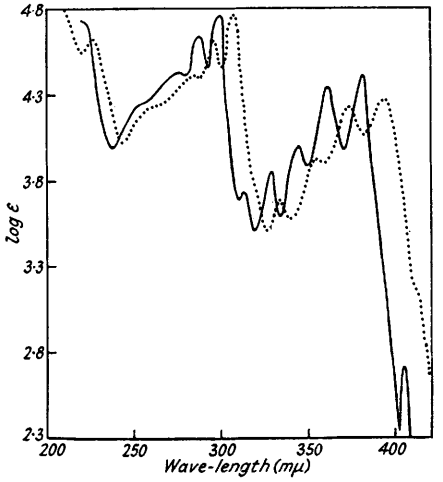
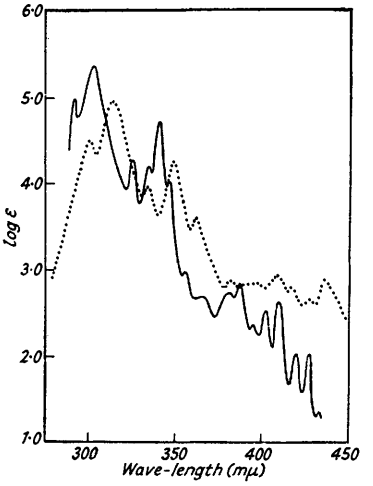


FIG. 2. *Absorption spectra of coronene* (——) *and the coronene fraction* (. . . . .) *from the zinc dust fusion of erythroaphin*-sl (*in* $CHCl_3$).



substance produced by a living organism. A substantial amount of smaller hydrocarbon fragments (probably both di- and tri-cyclic) were produced, but these were not examined further since they were obviously products of more extensive breakdown and were therefore less relevant to our immediate aim. The benzoperylene fraction gave a crystalline picrate from which the parent hydrocarbon could be regenerated but could not be crystallised and was obviously a mixture. Its analysis corresponded to that of a benzoperylene bearing 4—6 methyl groups or their equivalents. That it was a mixture of alkyl-1 : 12-benzoperylenes was also suggested by its ultra-violet absorption which showed a general bathochromic shift when compared with that of authentic 1 : 12-benzoperylene (Fig. 1). Such a shift is produced on introducing alkyl substituents into a polycyclic system (cf. Jones, *Chem. Reviews*, 1943, **32**, 1; Badger, Pearce, *et al.*, *J.*, 1951, 3072; 1952, 1112), its extent depending on their number and nature, as well as on their positions. Both the coronene fraction itself and the corresponding picrate were obtained crystalline and may well have been homogeneous, but the very small quantity obtained (much smaller than that of the perylene and the benzoperylene fractions) did not permit analysis. In this case, too, the ultra-violet absorption maxima of the hydrocarbon were shifted to longer wave-lengths than in authentic coronene, suggesting the presence of alkyl substituents (Fig. 2). The perylene fraction was likewise characterised by its absorption spectrum (Fig. 3); again

there was a shift indicating alkyl substituents; unfortunately chromatography failed to separate completely the benzoperylene impurity present in this fraction, nor could it be separated by recrystallisation of the picrate, so that no pure perylene derivative could be isolated in a crystalline condition.

Since the spectroscopic evidence indicates clearly the presence of the perylene, 1 : 12 benzoperylene, and coronene systems in the products of zinc dust fusion of erythroaphin-*sl*, we tried to obtain homogeneous individual hydrocarbons, in sufficient quantity to determine the nature and location of substituents, by reduction of erythroaphin with phosphorus and hydrogen iodide followed by distillation over copper (cf. Brockmann, " Progress in Organic Chemistry," Butterworth, London, 1952, Vol. I, p. 69); but, although the presence of benzoperylenes could be detected spectroscopically in the non-acidic fraction, the products appeared even more complex than those from the zinc dust fusions, and the method was not further pursued.

The experiments described in this paper provide strong support for the partial structure (I) for the erythroaphins, but the question arises whether the carbon skeleton of the original

FIG. 3. *Absorption spectra of perylene* (——) *and the perylene fraction* (. . . . .) *from the zinc dust fusion of erythroaphin*-sl (*in* 95% EtOH).

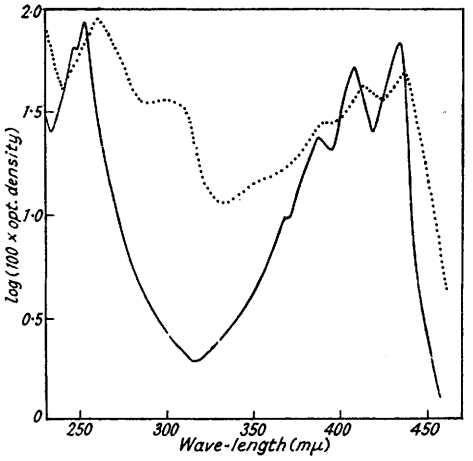
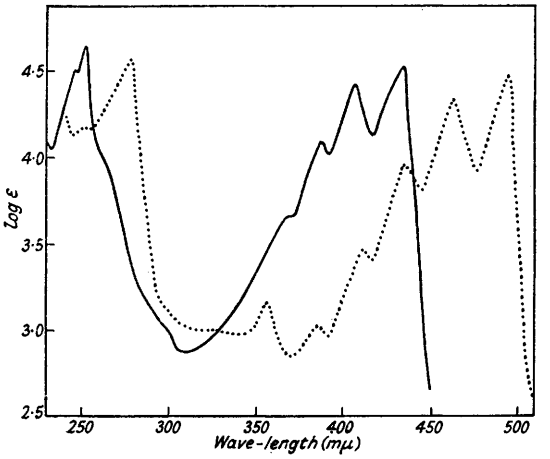


FIG. 4. *Absorption spectra of perylene* (——) *in* 95% EtOH *and of tetra-acetyldihydroerythroaphin*-sl (. . . . .) *in* EtOH *containing* 5% *of* $CHCl_3$.



pigment is that of perylene, 1 : 12-benzoperylene, or coronene, *i.e.*, whether the coronene fraction of the zinc dust fusion products is formed by ring closure or the benzoperylene and perylene fractions by ring fission during the reaction. Cyclisations involving suitably placed methyl groups in aromatic ring systems during high-temperature reactions have frequently been reported, *e.g.*, the production of anthrodianthrene from hypercin or 2 : 2′-dimethylnaphthodianthrene on zinc dust fusion (Brockmann, *loc. cit.*), of coronene from 9 : 12-dimethyldibenzophenanthrene-3-carboxylic acid by fusion with potassium hydroxide (Newman, *J. Amer. Chem. Soc.*, 1940, **62**, 1683), and of pyrene by heating of 4 : 5-dimethylphenanthrene with selenium at 300° (Newman and Whitehouse, *ibid.*, 1949, **71**, 3664). On the other hand the formation of small aromatic fragments by ring-fission is a well-known feature of high-temperature selenium dehydrogenations in, for example, the triterpene series (Jeger, *Fortschr. Chem. Org. Naturstoffe*, 1950, **7**, 15). Perylene, benzoperylene, and coronene all show high stability when fused with zinc dust under the conditions used in our experiments, a fact which suggests that in erythroaphin only the perylene system is present, *i.e.*, that the additional carbon atoms which give rise to the coronene and benzoperylene nuclei are not present in aromatic rings. This suggestion is strongly reinforced by a consideration of the absorption spectra of the tetra-acetyldihydroerythroaphins. In general the acetyl derivatives of reduced quinones have absorption spectra closely resembling those of the parent hydrocarbons, the acetoxy-groups causing only a weak

bathochromic shift (cf., *e.g.*, Brockmann, *loc. cit.*, p. 71). Tetra-acetyldihydroerythroaphin-*sl* (Part III, *J.*, 1950, 485) has an ultra-violet absorption spectrum which closely resembles that of perylene (Fig. 4) but is quite unlike those of benzoperylene and coronene. It is also significant that 3 : 4 : 9 : 10-tetrabenzoyloxyperylene (Pestemer, Schmidt, Schmidt-Wiligut, and Manchen, *Monatsh.*, 1938, **71**, 432) (*i.e.*, the benzoyl derivative from the undescribed 3 : 10-dihydroxyperylene-4 : 9-quinone) has a spectrum intermediate between those of perylene and tetra-acetyldihydroerythroaphin-*sl*.

Accepting this view, then to accommodate the results of the zinc dust fusion it must be assumed that in the erythroaphins *at least* one out of each pair of positions 1 : 12 and 6 : 7 in the perylene system of (I) must bear a saturated substituent attached through carbon. The evidence here presented does not permit decision between the various possibilities, *i.e.*, between a perylene with 2, 3, or 4 aliphatic side chains, a dihydrobenzoperylene with a side chain or chains at 6 or 7, and a derivative of 1 : 2 : 7 : 8-tetrahydrocoronene. These saturated portions of the molecule must accommodate the four unplaced oxygen atoms of erythroaphin, a fact which could, in any case, account for the very low yield of coronene derivative(s) obtained on zinc dust fusion.

Experimental

Solvents.—Benzene and light petroleum (b. p. 40—60°) used for the chromatography were purified by repeated shaking with concentrated sulphuric acid, until the acid was no longer discoloured, and then washed with water, dried ($CaCl_2$), and finally distilled and stored over sodium. Unless otherwise stated, light petroleum refers to the fraction of b. p. 40—60°.

Alumina was graded by the procedure of Brockmann and Schodder (*Ber.*, 1941, **74**, 73).

Ultra-violet absorption spectra were measured in benzene or 95% ethanol solution.

Zinc Dust Fusion of Erythroaphin-sl.—Erythroaphin-*sl* (20 mg.) was added to sodium chloride ("AnalaR"; 200 mg.) and zinc dust ("AnalaR"; 400 mg.). Zinc chloride (1 g.) was added and the mixture intimately mixed and transferred to a test-tube (6 × $\frac{5}{8}$") and fused at 215—220° for 10 min. and then at 290° for 15 min. in a salt-bath, with continuous stirring by a glass rod. The products of 250 such fusions were combined and digested with 3N-hydrochloric acid (1·2 l.), and the residue was separated, thoroughly washed with water, dried at 100°, and exhaustively extracted with benzene (Soxhlet). The benzene extract was shaken with 5% sodium hydroxide solution (4 × 125 c.c.) to remove phenols, washed with water, dried ($CaCl_2$), and concentrated to 200 c.c. before chromatography on a short column of grade III alumina (130 g.) (see Table). The column was developed with more benzene, and the following fractions were taken: Fraction A (350 c.c.) on evaporation gave a brown oil (561 mg.) which was subjected to further chromatography (see below). Fraction B (500 c.c.) gave a brown solid (82 mg.) after removal of solvent. Further chromatography of this fraction indicated that it was very similar to fraction A on the basis of ultra-violet spectra. Fraction C [250 c.c. of benzene–ethanol(10 : 1)] also gave a brown solid (184 mg.). Further chromatography gave fractions with indeterminate spectra which were not further investigated.

Fraction A was dissolved in benzene–light petroleum (1 : 2; 300 c.c.), brought on to a column of grade I alumina (2·5 × 30 cm.), and developed with the same solvent. Fractions were taken as shown in the scheme on p. 108, fractions 1—27 being 250 c.c. each, nos. 28—35 500 c.c., and nos. 36—42 1 l. each. For fractions 43—52 the solvent was changed to benzene (500 c.c. per fraction).

Perylene fraction. Fractions 5—21, which from the intensity of absorption at 440 mμ were estimated as containing 23 mg. of perylenes, were dissolved in benzene–light petroleum (1 : 3; 30 c.c.) and re-chromatographed on a column of grade I alumina (18 × 2 cm.). The column was developed with the same solvent and the fractions (250 c.c. each) shown in the scheme were taken. Fractions 2′—5′ had a spectrum very similar to that of the original mixture. So had fractions 6′—11′, except that the relative height of the peaks around 300 mμ was higher. It was evident that no further separation had been achieved in this experiment. In another attempt fractions 2′—5′ in benzene–light petroleum (1 : 5; 72 c.c.) were brought on to a column of mixed alumina–calcium hydroxide–Hyflo Supercel (3 : 1 : 1) (28 × 8 cm.) (Zechmeister and Koe, *Arch. Biochem.*, 1952, **35**, 1). The mixture was developed with the same solvent and, when the lowest fluorescent zone had reached the bottom of the column, the adsorbent was extruded and cut into eight fractions, each containing portions of the fluorescent zones. Each was eluted with benzene–ethanol (4 : 1), and the ultra-violet spectrum of each fraction determined. No marked separation was achieved. The fractions were combined, dissolved in

benzene, and converted into the picrate which formed brown needles. Reconversion of the picrate into the hydrocarbon on a short column of alumina gave a product which still contained benzoperylene.

Benzoperylene fraction. Fractions 25—42 (see scheme) were a greenish-yellow oil, estimated spectrophotometrically to contain 20 mg. of a benzoperylene. It was dissolved in hot benzene (8 c.c.), and picric acid (30 mg.) in benzene (1 c.c.) added. The brown crystalline picrate which separated (29 mg.) was dissolved in benzene (30 c.c.) and brought on to a small column of alumina to afford the hydrocarbon, which was sublimed at 0·1 mm. A trace of a clear non-fluorescent oil distilled first, which was rejected, but the main bulk distilled at 250° as a viscous greenish oil [Found : C, 93·7; H, 6·6. Calc. for $C_{22}H_8(CH_3)_4$: C, 93·9; H, 6·1. Calc. for $C_{22}H_6(CH_3)_6$: C, 93·3; H, 6·7%]. The remainder of the sample (estimated spectrophotometrically to contain 8·8 mg. of benzoperylene derivative) was dissolved in benzene, and picric acid (7·5 mg.) was added. The picrate crystallised as long reddish-brown needles which were re-converted into the hydrocarbon once again by the action of alumina and re-formed in benzene solution by the addition of picric acid (6 mg.). The *picrate* (10 mg.) slowly melted at 195° but was unstable at 100°. It was dried at room temperature/10^{-2} mm. [Found : C, 71·9; H, 5·5; N, 6·4. $C_{22}H_8(CH_3)_4,C_6H_3O_7N_3,C_6H_6$ requires C, 71·4; H, 4·6; N, 6·6. $C_{22}H_6(CH_3)_6,C_6H_3O_7N_3,C_6H_6$ requires C, 71·9; H, 5·0; N, 6·3%]. The ultra-violet absorption spectrum of the purified hydrocarbon in ethanol (95%) showed maxima at 395, 374, 356, 334, 308, 296, 285, and 227 mμ ($E^{1\%}_{1\,cm.}$ 462, 418, 210, 122, 1470, 1070, 660, and 1070 respectively) and an inflection at 258—262 mμ ($E^{1\%}_{1\,cm.}$ 425).

Coronene fraction. Fractions 43—52 (see scheme) were introduced in benzene–light petroleum (1 : 2; 90 c.c.) on to a column of grade I alumina and developed in benzene–light petroleum (1 : 1). Fraction A′ (1 l.) showed a typical benzoperylene spectrum. Fraction B′ (1·5 l.) was a mixture of benzoperylene and coronene derivatives, separated by sublimation and further chromatography (see below). Fraction C′ (2 l.) showed a typical coronene spectrum.

Fraction B′, after removal of solvent, was sublimed at 5×10^{-4} mm. At 80—140° a clear non-fluorescent oil was obtained, showing no marked absorption in the ultra-violet, and was therefore not further investigated. The residue was kept at 160°/10^{-4} mm. for 6 hr., a pale green oil distilling, the spectrum of which showed it to be a mixture of benzoperylene and coronene derivatives. The residue was a coronene derivative virtually free from benzoperylene and was combined with fraction C′. The mixture of benzoperylene and coronene derivatives obtained as the sublimate was brought in benzene–light petroleum (1 : 2; 75 c.c.) on to a column of alumina (Grade I) and developed with the same solvent. Twenty fractions were taken (12×250 c.c., then 8×500 c.c.), of which the first two exhibited a typical benzoperylene ultra-violet spectrum, and fractions 14—20 showed a typical coronene spectrum.

The combined coronene fractions (fraction C′ + the unsublimed residue from fraction B′ + the coronene obtained in the sublimate from fraction B′) were estimated spectrophotometrically to contain 1·4 mg. of coronene derivative, which after removal of the solvent formed a yellowish viscous oil. It was dissolved in a little benzene, a solution of picric acid (1·2 mg.) in benzene added, and the mixture warmed. After concentration and cooling the picrate crystallised as small reddish rods which were separated (centrifuge) and recrystallised from benzene. The picrate slowly melted at >200° with decomposition. It was re-converted in benzene solution into the parent hydrocarbon on a small column of alumina, and the product sublimed at 200°/0·1 mm. as a yellow semi-solid mass. This was made into a mull in Nujol for infra-red examination (below) and subsequently washed into a small test-tube with benzene. Removal of the benzene gave a concentrate from which clusters of yellow needles slowly separated. The crystals (0·2 mg.) were washed with light petroleum and dried at 100°/14 mm. The ultra-violet absorption spectrum (Fig. 2) of a solution in benzene showed maxima at 363, 351, 335, 315, and 302 mμ. The infra-red spectrum showed distinct maxima at 6·20, 7·70, and 11·33 μ. For comparison the infra-red spectrum of coronene was determined as a mull in Nujol, the following maxima being observed : 6·23, 7·62(s), 10·45, 11·78(s), and 13·0 μ.

This work was carried out during the tenure of an 1851 Senior Studentship by B. R. B., and of a D.S.I.R. Senior Award by J. R. Q. We are deeply indebted to Dr. Otto Bayer of Farbwerke Leverkusen for generous samples of coronene, Imperial Chemical Industries Limited, Dyestuffs Division, for gifts of perylene and intermediates for its preparation, and to Professor J. W. Cook for an authentic specimen of 1 : 12-benzoperylene.

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