

Fig. 2

In a given experiment, cultures grown in the klinostat tubes were not only larger and more uniform but also, under the standardized conditions of the klinostat, the growth-rates of carrot cultures are much more reproducible than when the cultures are grown in nutrient agar in the test tubes.

Fig. 2 shows a growth-curve of cultures of carrot initially 4 mgm. in weight. At each period four replicate cultures were weighed, and the curve is compiled from their means. There is evidently a lag of about four days followed by a very rapid growth reaching a compound-interest rate of increase in twelve days of 34.1 per cent per day. After some days a gradual decrease in the growth-rate occurs. This decrease is probably due in part to depletion of nutrients in the culture medium. (Changing the culture solution after some fourteen days does increase the growth.)

Single cultures readily grow from a 4-mgm. explant to about 175 mgm. before they become so large that they do not readily adhere to the glass, and so fall as the tube rotates. The amount of tissue grown in a given tube and medium may be increased by using more explants per tube, but this markedly reduces the size of each cultured explant. For the most part, the technique has been applied to cultures freshly excised from the carrot root. The rates of growth of subcultures tend to be more variable, probably because they cannot readily be cut into pieces of uniform size and shape which contain identical proportions of active cells.

Cultures grown in liquid media in klinostat tubes and on agar differ in form: the former become compact spheroids, especially when grown in the light, while those on agar become much more succulent and irregularly concave to the medium. In the dark, cultures in klinostat tubes become friable and tend to fall apart. These differences are being investigated and will be reported elsewhere. In the dark, carrot phloem cultures change from deep orange to pale. In the light they become pale green. Cultures from certain carrots in the light have, however, been observed to become unpredictably coloured with a red pigment in the surface layers of cells. This does not, however, affect the conclusions on the efficacy of the technique here described.

Clearly this technique is applicable to the growth of other materials in liquid media. The growth of *Chlorella vulgaris* in the klinostat tubes was found to compare very favourably with growth under other conditions in liquid media as used by other workers. Ten ml. of the basal culture solution, in equilibrium with the carbon dioxide of the air, inoculated to contain 75 cells per mm.³, produced in 7 days at 25° C. 57,000 cells per mm.³ at a light intensity of 240 foot-candles. The technique may well find application in the experimental growth of fungi for antibiotic studies, growth studies of yeast, etc. It has, however, proved its use in quantitative investigations on the growth of plant tissue cultures.

¹ White, P. R., *Bull. Torrey Bot. Club.*, **66**, 507 (1939).

² DeRopp, R. S., *Science*, **104**, 371 (1946).

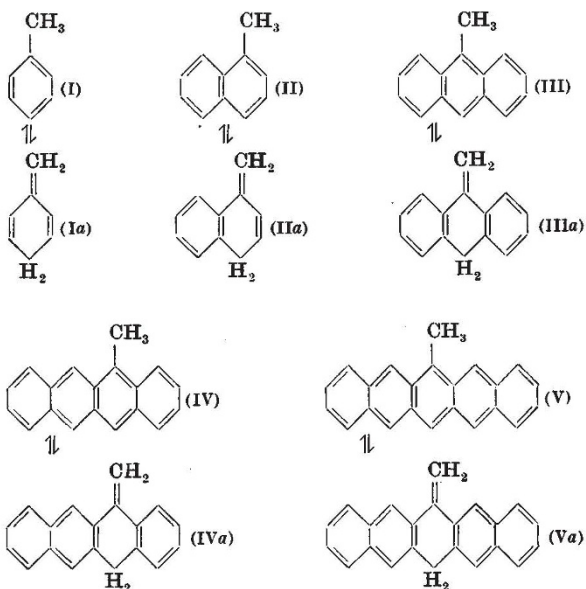
³ Caplin, S. M., and Steward, F. C., *Science*, **108**, 655 (Dec. 1948).

⁴ White, P. R., "A Handbook of Plant Tissue Culture" (Jaques Cattell Press, Lancaster, Pa.).

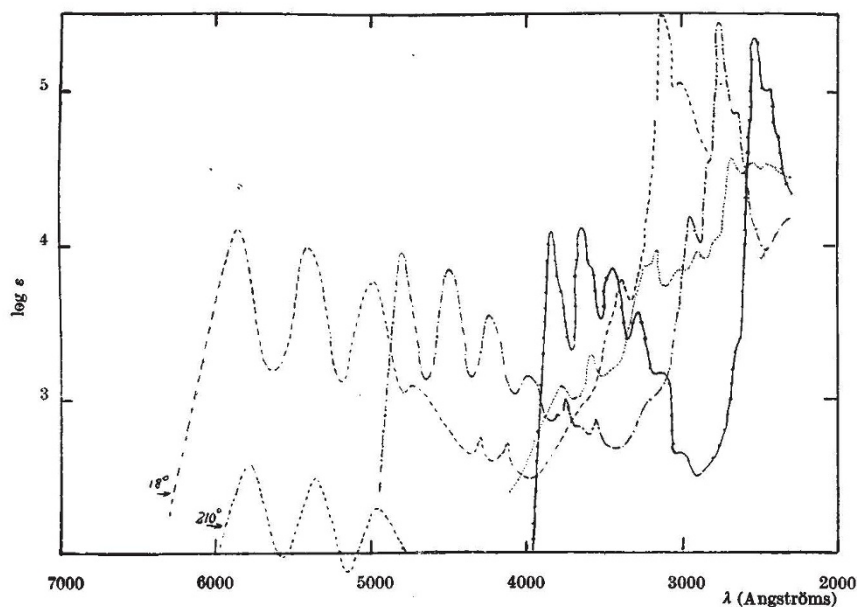
EQUILIBRIUM BETWEEN METHYL-ACENES AND METHYLENE-DIHYDROACENES

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TAUTOMERISM between methyl-acenes (I, II, III, IV, V) and methylene-dihydroacenes (Ia, IIa, IIIa, IVa, Va, respectively) has often been postulated to explain side-chain reactivity of aromatic compounds, but hitherto no methylene-dihydroacene has been isolated. This weakness in the hypothesis has now been removed by the synthesis of pure 6-methylene-6:13-dihydropentacene (Va). The 'anellation principle'¹ requires a gradual transition in properties in the acene series, and it may be inferred, therefore, that (IV), (III), (II) and (I) will also contain the corresponding methylene forms, but in decreasing proportions.



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- Absorption spectrum of 9-methylanthracene in alcohol. Band maxima at : 3850, 3640, 3460, 3290, 3140, 3000 ; 2540, 2460 A.
- Absorption spectrum of 5-methyltetracene in alcohol. Band maxima at : 4805, 4490, 4220, 3980 ; 3750, 3560 ; 2950, 2755, 2650 A.
- Calculated absorption spectrum of 6-methylpentacene in 1-methylnaphthalene. Band maxima at : 5850, 5410, 5010 ; 4300, 4120 ; 3390, 3150 A.
- · - · - (Lower curve.) Observed absorption of methylpentacene at 210° in 1-methylnaphthalene : 5790, 5360, 4960 A. (intensity qualitative); on rapid cooling the fading band maxima shift to : 5850, 5410, 5010 A.
- Absorption spectrum of 6-methylene-6 : 13-dihydropentacene (Va) in alcohol. Band maxima : 3770, 3585, 3420 ; 3175, 3020, 2900 ; 2690, 2570 A.

This view is supported by study of the absorption spectra of the hydrocarbons (see accompanying graph). The spectrum of 9-methylanthracene (III) resembles very closely that of anthracene, but does not exclude the presence of a small proportion of the methylene form (IIIa), which would have only feeble absorption. The orange 5-methyltetracene (IV) shows an absorption similar to that of tetracene, but the intensity of the bands is considerably lower than in the case of tetracene, whereas with 9-methylanthracene and anthracene the reverse relationship holds. This suggests the presence in (IV) of an appreciable proportion of a weakly absorbing methylene form (IVa).

When we come to the pentacene series, we find that the spectrum of the pale yellow methylene-dihydropentacene (Va) shows no resemblance to that to be expected for methylpentacene (V), which may be calculated by application of the anellation principle¹. If the pale yellow solution of (Va) in 1-methylnaphthalene is heated with exclusion of air to 200°, the solution becomes violet-red and shows the first three bands calculated for methylpentacene (V). The colour fades slowly on cooling.

The fact that methylpentacene exists at room temperature almost entirely in the methylene form (Va) is important from the point of view of resonance in aromatic compounds. As each member of the pairs of structures formulated above contains the

same number of double bonds as the other member, the energy-difference between the methyl form and the methylene form must consist of the difference in resonance energy. Wave mechanical treatment predicts a decreasing difference in resonance energy of these pairs of hydrocarbons with increasing numbers of rings, until the difference becomes zero with an infinite number of rings². Hence the ratio between methylene and methyl forms should never exceed 1 : 1. This prediction has thus been shown to be entirely erroneous, and the evidence now adduced may perhaps provide a new basis for the calculation of the decreasing energy per ring in the higher acenes.

9-Methylanthracene (III) was prepared as described by Sieglitz and Marx³. 5-Methyltetracene (IV) was obtained by dehydration of the crude carbinol formed by interaction of methylmagnesium iodide with tetracenone, prepared by reduction of 5 : 12-tetracenequinone⁴ with alkaline sodium hydrosulphite solution. It formed orange-red prisms (from benzene), m.p. 160°, and gave a bright green solution in sulphuric acid (found : C, 94.2 ; H, 5.7. C₁₉H₁₄ requires C, 94.2 ; H, 5.8 per cent).

6-Methylene-6 : 13-dihydropentacene (Va) was similarly prepared from pentacenone⁵, and formed pale yellow needles (from xylene), which dissolved in sulphuric acid to give a blue solution which afterwards turned to brown (found : C, 94.4 ; H, 5.65. C₂₃H₁₆ requires C, 94.5 ; H, 5.5 per cent). The melting point was between 244° and 254°, and in an evacuated capillary the melt was deep red. Oxidation with selenium dioxide in boiling nitrobenzene gave pentacene-5 : 13-quinone. A sparingly soluble by-product from the Grignard condensation formed colourless crystals, m.p. 420° (decomp.), which gave a greenish-yellow solution in sulphuric acid (found : C, 94.2 ; H, 5.8. C₁₆H₁₂ requires C, 94.5 ; H, 5.5 per cent).

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¹ Clar, E., "Aromatische Kohlenwasserstoffe", 20 (Springer-Verlag, 1941).

² Sirkin, J. K., and Diatkina, M. E., *Bull. Acad. Sci. U.R.S.S., Cl. Sci. Chim.*, 153 (1946). *Brit. Abstr.*, A1, 365 (1946). The view of these authors was supported, and in some cases an even greater stability of the higher methyl-acenes was predicted, in private communications from other workers.

³ *Ber.*, 56, 1619 (1923).

⁴ Clar, E., *Ber.*, 75, 1271 (1942). Compare Fieser, L. F., *J. Amer. Chem. Soc.*, 53, 2329 (1931).

⁵ Marschalk, Ch., *Bull. Soc. Chim.*, (5), 4, 1549 (1937).