789. *Colouring Matters of the Aphididæ. Part XV.* The Alkaline Inversion of Erythroaphin-*sl *and its Derivatives.*†

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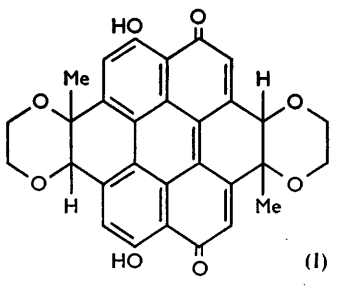
Solvent systems are described for the paper chromatographic analysis of mixtures of aphin pigments and particularly for the differentiation of *fb* and *sl* isomers. Silica columns have been used for larger-scale separations. Erythroaphin-*fb* has been isolated from the products of reaction of alkali with erythroaphin-*sl*, and hydroxyerythroaphin-*fb* from the reaction of erythroaphin-*sl* with sodium periodate.

In an earlier paper [1] structure (I) was advanced for erythroaphin, the relatively stable, deep red aphin pigment. Two isomers of erythroaphin have been obtained from the protoaphins of different aphid species, the glucosidic water-soluble protoaphins being converted successively into xanthoaphins, chrysoaphins, and erythroaphins. The difference between these two isomers, erythroaphin-*fb* and erythroaphin-*sl*, has been shown to be stereochemical on the grounds of the close similarity of chemical and light-absorption properties and the methods by which erythroaphin-*sl* can be converted into the -*fb* isomer. Amination or hydroxylation of erythroaphin-*sl* gave the diamino- or dihydroxy-derivative of erythroaphin-*fb*, and reduction of these substitution products with zinc and acetic acid yielded erythroaphin-*fb* itself.[2] Ultraviolet irradiation of either tetra-acetyldihydro-erythroaphin-*fb* or -*sl* caused racemisation and an equilibrium mixture was obtained containing the two isomers together with a third isomer derived from an erythroaphin which has not, as yet, been obtained from aphid species.[3] It was deduced that the ring junctions between the two sets of non-aromatic rings were *cis-cis* for erythroaphin-*fb*, *cis-trans* for erythroaphin-*sl*, and that the new artificially produced erythroaphin was the *trans-trans*-isomer. The arguments leading to these conclusions depended on the quantitative determination of acetaldehyde liberated from the erythroaphin-*fb* and -*sl* on treatment with strong acids, as well as stereochemical considerations of the course of the addition reactions.



(I)

Direct conversion of the less stable erythroaphin-*sl* into the -*fb* isomer by the action of alkali has not hitherto been accomplished, partly because of the difficulty of recognising small quantities of one isomer in the presence of the others and partly because of the ready nuclear hydroxylation which occurs in alkaline solution. Solvent systems have now been

* Part XIV, *J.*, 1955, 1144.
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[1] Brown, Calderbank, Johnson, Joshi, Quayle, and Todd, *J.*, 1955, 959.
[2] Brown, Calderbank, Johnson, MacDonald, Quayle, and Todd, *J.*, 1955, 954.
[3] Brown, Calderbank, Johnson, Quayle, and Todd, *J.*, 1955, 1144.

discovered which permit the analysis of such reaction products by chromatography on paper; and, for larger quantities, columns of silica, deactivated by the addition of small amounts of water, have been used successfully. It has thus been found that the action of hot 0·5% aqueous sodium hydroxide on erythroaphin-*sl* is complex and gives in the course of 30 minutes at least five products; however, crystalline erythroaphin-*fb* has been isolated in about 10% yield from the mixture. Apart from insoluble black materials, the other compounds formed appeared to be mono- and di-hydroxy-derivatives of erythroaphin.

A similar experiment with erythroaphin-*fb* was superficially the same but none of the -*sl* isomer was found among the products. The ease with which the inversion of erythroaphin-*sl* and its derivatives occurred is emphasised by the formation of hydroxyerythroaphin-*fb* as well as the -*sl* isomer when an aqueous-dioxan solution of erythroaphin-*sl* is heated under reflux for 30 hours. Pure hydroxyerythroaphin-*fb* was also obtained by heating erythroaphin-*sl* with a dilute solution of sodium periodate. Paper-chromatographic evidence has also been obtained for the formation of erythroaphin-*fb* from erythroaphin-*sl* by the action of hot dilute sulphuric acid.

Cyclic compounds containing an activated hydrogen atom at a ring junction are known to be converted into the more stable isomers by alkali or acids, and examples are common among steroids[4] and related compounds;[5] the direct conversion of erythroaphin-*sl* into erythroaphin-*fb* is most readily explained by this type of mechanism. In Part XIV of this series the interconversion of erythroaphin derivatives was discussed. At the time that paper was written we had no examples of conversion from the *sl* into the *fb* series, in which the reactions involved did not include reduction and reoxidation accompanied by the introduction of at least two substituents. Consequently the explanations suggested were based largely on considerations of steric hindrance. The results recorded in the present memoir show that direct conversion of erythroaphin-*sl* into erythroaphin-*fb* can be effected by treatment with alkali, or, less readily, acid. This being so, steric hindrance, although it may be a factor in some reactions, is much less important than was suggested in Part XIV and may indeed be of minor significance. Basic reagents bring about inversion rapidly and it is therefore almost certain that inversion occurs with the introduction of the first amino- or hydroxy-group during formation of diamino- and dihydroxy-erythroaphins. Inversion with acid is much slower than with alkali, a fact which is of itself sufficient to explain the apparent lack of inversion during the Thiele acetylation and bromination of erythroaphin-*sl*.

Experimental

Chromatography of the Aphin Pigments.—Best results were obtained by using the organic layers of "AnalaR" chloroform–light petroleum (b. p. 100—120°)–water mixtures. For general use these solvents were used in the proportion 2 : 2 : 1 respectively; for xanthoaphins and chrysoaphins the proportion 4 : 1 : 1 was preferred; for the hydroxy-erythroaphins 10 : 5 : 3, and for the fast-moving erythroaphins the ratio 3 : 7 : 2, was employed. Reproducible R_F values were obtained with Whatman No. 4 paper in ascending chromatograms when (i) a small tank (30 × 8 cm.) was used, (ii) the solvent was replaced after each chromatogram, (iii) the minimum amount of pigment was used (a spot which was just visible was sufficient) and (iv) each chromatogram was run for approximately 1 hr., during which the solvent front moved 15—18 cm. When the organic layer of the chloroform–petroleum–water (2 : 2 : 1) mixture was used, typical R_F values were: erythroaphin-*sl*, 0·93; erythroaphin-*fb*, 0·78; hydroxyerythroaphin-*sl*, 0·14; hydroxyerythroaphin-*fb*, 0·14; chrysoaphin-*sl*, 0·53, 0·44, and 0·33 (indicating different isomers); chrysoaphin-*fb*, 0·18; xanthoaphin-*sl*, 0·08 and 0·02 (different isomers); and xanthoaphin-*fb*, 0·02. With the same solvent mixture in the ratio 4 : 1 : 1, the following values were obtained: xanthoaphin-*sl*, 0·53 and 0·28; chrysoaphin-*fb*, 0·65; xanthoaphin-*fb*, 0·12. Discussion of isomerism among xanthoaphins and chrysoaphins is deferred until further investigation of its nature is complete. Faint spots were best detected in ultraviolet light.

Chromatography on Silica.—Good results were obtained with silica gel (40-mesh) containing 10% by wt. of water and equilibrated by rolling for 24 hr. The column was packed by using

[4] See, for example, Crawshaw, Henbest, Jones, and Wyland, *J.*, 1955, 3420.
[5] See, for example, Walker, *J.*, 1954, 3960.

1 : 1 benzene–light petroleum (b. p. 60—80°), and a benzene solution of the substance was brought on the column. Elution was effected with benzene and then with benzene–propan-2-ol mixtures. For aphins which are very strongly absorbed, *e.g.*, dihydroxyerythroaphin, the silica–water proportion was raised to 20 : 3, and pressure was sometimes necessary to ensure movement through the column.

Action of Alkali on Erythroaphin-sl.—1% Aqueous sodium hydroxide (200 c.c.) was added to a solution of erythroaphin-*sl* (400 mg.) in dioxan (200 c.c.), and the mixture heated under reflux for ½ hr. The product was cooled and acidified with 3N-hydrochloric acid, and the solution thoroughly extracted with ether; the insoluble black material which formed at the interface was discarded. The ethereal extract was washed and dried, the solvent removed, and the residue dissolved in " AnalaR " benzene (200 c.c.). The benzene solution was chromatographed on silica gel–water (9 : 1; 65 g.), and the following fractions were taken, benzene containing increasing proportions of propan-2-ol being the eluant: (1) benzene containing 0·25% of propan-2-ol (400 c.c.) + benzene containing 0·5% of propan-2-ol (100 c.c.) (70 mg.); (2) benzene containing 0·5% of propan-2-ol (100 c.c.) (1 mg.); (3) benzene containing 0·5% of propanol-2-ol (400 c.c.) + benzene containing 2% of propan-2-ol (100 c.c.) (67 mg.); (4) benzene containing 2% of propan-2-ol (500 c.c.) (46 mg.). Fraction (1) was crystallised from chloroform–ethanol [6] and gave erythroaphin-*fb* (42 mg.) (Found: C, 70·4; H, 4·4. Calc. for $C_{30}H_{22}O_8$: C, 70·6; H, 4·35%). The identity of the product was confirmed by chromatography and determination of its ultraviolet and infrared spectra.[7] No crystalline products were isolated from fraction 3 or 4 but chromatography suggested the presence of hydroxy-derivatives of erythroaphin.

Action of Water on Erythroaphin-sl.—Hot water (50 c.c.) was added to a solution of erythroaphin-*sl* (100 mg.) in freshly purified dioxan (100 c.c.), and the solution heated under reflux for 30 hr. It was then cooled, diluted with an equal a volume of water, and acidified with 3N-hydrochloric acid. The solution was extracted with ether (3 × 150 c.c.) and then chloroform (100 c.c.), and the combined extracts were washed, dried (Na_2SO_4), and evaporated. The residue was dissolved in " AnalaR " benzene (100 c.c.) and chromatographed on silica gel (15 g.; containing 3 parts of water to 20 parts of silica). The following fractions were taken: (i) benzene (100 c.c.) (10 mg.); (ii) benzene (300 c.c.) (40 mg.); (iii) benzene (200 c.c.) (6 mg.); (iv) benzene containing 0·5% of propan-2-ol (300 c.c.) (15 mg.). Each fraction was investigated by paper chromatography: fraction (i) appeared to be essentially unchanged erythroaphin-*sl*, and fraction (ii) a mixture of monohydroxy-derivatives. Fraction (ii) was crystallised from chloroform–ethanol, and the first crop (7 mg.) recrystallised from the same solvent. The crystalline product (3 mg.) was a hydroxyerythroaphin-*sl* which yielded erythroaphin-(*sl*?) after reduction with zinc and acetic acid. The second crop (13 mg.) from the crystallisation, on recrystallisation proved to be a mixture of the isomers of hydroxyerythroaphin (chromatography and infrared spectrum). Reduction with zinc and acetic gave erythroaphin-*fb* containing a small quantity of the -*sl* isomer.

Action of Sodium Periodate on Erythroaphin-sl.—A hot solution of sodium periodate (50 mg.) in water (50 c.c.) was added to a solution of erythroaphin-*sl* (100 mg.) in dioxan (50 c.c.), and the resulting suspension heated under reflux for 6 hr. Dioxan (50 c.c.) was then added to effect complete dissolution and the heating continued for a further 8 hr. The volume of the solution was reduced to 100 c.c., and the mixture heated under reflux for a further 6 hr., after which it was established by chromatography that very little unchanged erythroaphin remained. The product was extracted into benzene as described in the previous experiment and the benzene solution put on a column of silica gel (15 g.; containing water as above) packed with 1 : 1 benzene–light petroleum (b. p. 60—80°). Hydroxyerythroaphin and any unchanged erythroaphin were not absorbed on the column but the dihydroxyerythroaphins were retained and could be eluted with benzene containing 0·5% of propan-2-ol. The hydroxyerythroaphin fraction (40 mg.) was purified by crystallisation from chloroform–ethanol, being obtained as red needles (Found: C, 67·9; H, 4·2. Calc. for $C_{30}H_{22}O_9$: C, 68·4; H, 4·2%). The infrared spectrum and the chromatographic behaviour of the product were identical with those of authentic hydroxyerythroaphin-*fb*. Reduction of the product with zinc and acetic acid [6] gave erythroaphin-*fb* containing a trace only of the -*sl* isomer (chromatography).

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[6] Brown, Johnson, MacDonald, Quayle, and Todd, *J.*, 1952, 4928.
[7] Johnson, Todd, *et al.*, *J.*, 1950, 477; 1951, 2633.