960. *Colouring Matters of the Aphididæ. Part VII.* Addition Reactions of Erythroaphin-*fb.

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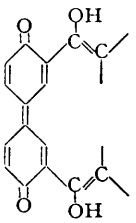
Erythroaphin-*fb* readily undergoes addition reactions with ammonia, amines, and halogens with the formation of diamino-, substituted diamino-, and dihalogeno-erythroaphins. Diaminoerythroaphin-*fb* is converted by nitrous acid into dihydroxyerythroaphin-*fb* which is also formed by oxidation of erythroaphin-*fb* with permanganate. Thiele acetoxylation of erythroaphin followed by hydrolysis yields monohydroxyerythroaphin. All of these substitution products of erythroaphin can be reconverted into the parent pigment by reduction with zinc and acetic acid. The bearing of these reactions on the structure of erythroaphin is discussed.

Earlier work has shown that the erythroaphins from various aphid species are polycyclic quinones and by combining it with evidence from spectroscopic studies it was concluded in Part V (Johnson, Quayle, Robinson, Sheppard, and Todd, *J.*, 1951, 2633) that they must be extended quinones (*i.e.*, compounds in which the quinone carbonyl groups are in different rings) containing two hydroxyl groups so situated that they can form with the carbonyl groups chelated 6-membered rings. Thus the simplest explanation of the facts

* Part VI, preceding paper.

was given by the presence of the grouping (I) in the erythroaphin molecule. The present paper provides chemical evidence which supports and extends this conclusion.

In an investigation of primitive aphids of the *Hamamelistes* family (forthcoming publication), certain pigments (rhodoaphins) were isolated which although not identical with any of the known erythroaphins appeared to be closely related to or derived from them, and some experiments were undertaken on derivatives of erythroaphin in the hope of establishing such a relation. In this way the remarkable observation was made that when erythroaphin-*fb* dissolved in aqueous dioxan was treated with ammonia in presence of air at 70—100° it was rapidly converted into a beautifully crystalline diaminoerythroaphin-*fb*; this product was quite strongly basic and gave a crystalline dihydrochloride. Diaminoerythroaphin-*fb* crystallised as a hydrate and analysis gave values corresponding to a formula $C_{30}H_{24}O_8N_2$, *i.e.*, to a diamino-derivative of erythroaphin, $C_{30}H_{22}O_8$. It may be recalled that a decision between $C_{30}H_{24}O_8$ and $C_{30}H_{22}O_8$ cannot readily be taken solely on the analytical values obtained for erythroaphin-*fb* itself (Part II, *J.*, 1950, 477). The reaction leading to the formation of diaminoerythroaphin-*fb* requires careful control of temperature, water–dioxan ratio, and particularly the ammonia–air ratio if a reasonable yield of crystalline product is to be obtained and uncrystallisable by-products are to be avoided. Similar derivatives are obtained when erythroaphin-*fb* is treated with a number of other amines in presence of air, and dipiperidino- and di*cyclo*hexylamino-erythroaphin-*fb* have been prepared in this way. When treated with nitrous acid diaminoerythroaphin-*fb* does not form a stable diazonium compound but is converted directly to dihydroxyerythroaphin-*fb*, $C_{30}H_{22}O_{10}$; some evidence was obtained for the existence of an intermediate compound, possibly a hydroxy-aminoerythroaphin-*fb* when insufficient nitrous acid was used. The same conversion into the dihydroxy-derivative can be effected by hot dilute mineral acid, or a mixture of acetic and boric acids at 100°. Dihydroxyerythroaphin-*fb* has also been obtained in low yield together with other products by oxidising erythroaphin-*fb* with potassium permanganate. The formulation of the dihydroxy-compound is justified not only by its composition and mode of formation but also by the fact that it can be reduced, like diaminoerythroaphin-*fb*, by short treatment with zinc and acetic acid at 100° to erythroaphin-*fb*, identified by analysis and by visible and infra-red spectra. Erythroaphin-*fb* is also very sensitive to halogens and dichloro- and dibromo-erythroaphin-*fb* are readily obtained by treatment of a chloroform solution with chlorine water or by the action of bromine in acetic acid respectively. The dihalogenoerythroaphins are also readily reduced to the parent erythroaphin-*fb* by zinc and acetic acid. During this work it was found that certain impurities may be conveniently removed from erythroaphin-*fb* by shaking its chloroform solution with saturated sodium hydrogen carbonate solution, the impurities being precipitated at the interface. This step has been included in all of the erythroaphin preparations.


(I)

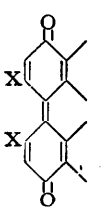
These reactions of erythroaphin-*fb* find their closest analogy in the known behaviour of quinones. The amination reaction on this basis may be assumed to proceed by initial addition of ammonia or other amine to the quinonoid system, the quinol derivative first formed being reoxidised by air to an aminated erythroaphin which then undergoes a second additon reaction. The conversion of diamino- into dihydroxy-erythroaphin-*fb* has a parallel in the behaviour of a number of aminoquinones, and the production of dihydroxyerythroaphin on permanganate oxidation is presumably due to the initial phase of oxidation (*i.e.*, hydroxylation) being markedly faster than the second phase so that provided insufficient permanganate is used to bring about complete degradation some dihydroxyerythroaphin will always be found among the products. Although these reactions of erythroaphin are typical of quinones the ease with which they occur is most surprising (*e.g.*, dipiperidinoerythroaphin-*fb* can be obtained by merely keeping erythroaphin-*fb* in an atmosphere of piperidine at room temperature). Amination with ammonia or amines is known and has been employed technically with a number of polycyclic hydroxyquinones but in all cases recorded in the literature the temperature required to bring about reaction is much higher than in the present instance and normally results in the replacement of hydroxy- by amino-

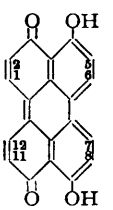
groups. The ease of reaction observed is much more reminiscent of a simple benzoquinone or naphthaquinone although even there it would be rather remarkable. There is another respect too in which the erythroaphins show the high reactivity of simple quinones; they appear to undergo the Thiele acetylation reaction. Thus treatment of erythroaphin-*fb* at 0° with acetic anhydride containing sulphuric acid, followed by decomposition of the excess of acetic anhydride, hydrolysis, and aerial oxidation, yields a hydroxyerythroaphin-*fb* which can be reconverted into erythroaphin-*fb* by reduction with zinc and acetic acid or with alkaline dithionite followed by aerial oxidation. It should be mentioned that we have so far been unable to introduce further hydroxyl groups into hydroxy- or dihydroxy-erythroaphin and or to aminate hydroxyerythroaphin under the conditions used for erythroaphin itself. It is known (cf. Thiele and Winter, *Annalen*, 1900, **311**, 341) that the presence of a 2-hydroxy or a 2-methyl group in naphthaquinone retards the normal Thiele acetylation reaction. The striking colour reaction for aphin pigments first noted by Blount (*J.*, 1936, 1034) in the case of strobinin (erythroaphin-*st*) can now be explained. The production of a blue solution with red fluorescence when aphins are treated with acetic anhydride containing sulphuric acid is due to Thiele acetylation of the erythroaphin. Tetra-acetyldihydroerythroaphin does not give a blue colour with concentrated sulphuric acid. The blue colour is characteristic of the acetates of the hydroxylated dihydroerythroaphins in strong acids; in neutral solution they are yellow. As would be expected on this basis the speed with which the colour develops increases with increasing hydroxylation and while acetic anydride–sulphuric acid and acetic anhydride–perchloric acid are suitable reagents for erythroaphin and hydroxyerythroaphin, acetic anhydride–phosphoric acid which has no effect on erythroaphin is the best reagent for use with dihydroxyerythroaphin.

The properties of the new compounds differ from those of erythroaphin in accordance with expectation. The introduction of hydroxy-groups progressively increases solubility in hydroxylic solvents and their acidity as reflected in the pH at which green alkali salts are formed; dihydroxyerythroaphin frequently develops a green colour with commercial methanol or acetone. Their visible absorption spectra, although quite distinct, are similar to that of erythroaphin but the compounds can be readily distinguished from erythroaphin and from each other by partition between various solvents (see Experimental).

All the present evidence indicates that the erythroaphins are polycyclic quinones. A simple benzoquinone or even naphthaquinone structure for them is virtually excluded and yet there are no analogies in the literature relating to polycyclic quinones for the remarkable ease of amination and hydroxylation; this appears to us to indicate that the quinonoid carbonyl groups are in peripheral rings of the polycyclic system. The fact that two amino-groups are introduced with ammonia suggests that the carbonyl groups occur in different rings, *i.e.*, that in the erythroaphin molecule we have an extended quinone system.

The simplest expression of this hypothesis would be the partial structure (II) but on steric grounds it is unlikely that amino-, hydroxy-, or halogeno-groups could be introduced into both of the positions X (cf. Newman and Whitehouse, *J. Amer. Chem. Soc.*, 1949, **71**,

(II) 

 (III)

3664). The structure (I) suggested on spectroscopic grounds has accordingly been developed to (III) where the tautomerism of the *peri*-dihydroxyquinone system would presumably permit the introduction of two amino-groups, etc., at positions 1 : 7 or 6 : 12. The perylene ring system would also provide mellitic acid in the nitric acid oxidation (Part II, *loc. cit.*).

These views rest, of course, on the assumption that the introduction of amino-groups, etc., proceeds by a normal 1 : 4-addition process. In view of the recent work of Adams and Holmes (*J. Amer. Chem. Soc.*, 1952, **74**, 3033, 3038) it is also possible that 1 : 8-addition

might occur in such a system; if this were so, there would presumably be much less steric resistance to the introduction of groups at positions 2, 5, 8, or 11 in (III). It is unfortunate that no work seems to have been done on the addition reactions of quinones of this type, *e.g.*, the perylene-3 : 10-quinones. We are accordingly investigating the structural features necessary for such ready addition, and the results will be reported later.

Experimental

Diaminoerythroaphin-fb.—A solution of erythroaphin-*fb* (505 mg.) in purified dioxan (200 c.c.) containing water (20 c.c.) and quinol (1·25 g.) was kept at 70° under reflux while a slow stream of ammonia and a faster stream of air were bubbled through it. After 1½ hours the mixture was cooled and poured into light petroleum (b. p. 40—60°; 800 c.c.). The green aqueous layer was separated, washed with more petroleum, and acidified with hydrochloric acid (150 c.c. of 10%). The red solution was filtered, diluted with water (150 c.c.), cooled, and partially neutralised with sodium hydroxide solution (100 c.c. of 10%). The pigment was then extracted into chloroform (100 c.c.; then 3 × 50 c.c.) and purified by extraction from the filtered chloroform solution into hydrochloric acid (3 × 25 c.c. of 10%), and then back again into chloroform (100 c.c.; then 2 × 50 c.c.) after dilution with ice and water and addition of sodium hydroxide solution (50 c.c. of 10%). When the filtered chloroform solution was concentrated and the volume maintained by addition of ethanol, *diaminoerythroaphin*-fb (212 mg.) separated as small crystals. These were dissolved in chloroform (50 c.c.) and shaken for 18 hours with saturated sodium hydrogen carbonate solution. The precipitate, largely consisting of impurities, was separated and washed with a little chloroform and the combined chloroform solutions were dried and concentrated, as before, with gradual addition of ethanol, to yield the product as deep red plates. For analysis it was recrystallised from the same solvents and dried for 6 hours at 110°/2 × 10^{-4} mm. (Found: C, 64·5; H, 4·9; N, 4·9. $C_{30}H_{24}N_2O_8,H_2O$ requires C, 64·5; H, 4·7; N, 5·0%). Light absorption in chloroform containing 5% of ether: Max. at 595, 567, 526, 489, 454, 429, 335, and 255 mμ; log ε 3·77, 4·23, 4·05, 3·76, 4·55, 4·42, 3·63, and 4·56 respectively. The infra-red spectrum of diaminoerythroaphin-*fb* as a mull in Nujol showed maxima at 697, 722, 762, 775, 797, 833, 855, 921, 980, 996, 1017, 1053, 1063, 1080, 1161, 1205, 1236, 1290, 1575, and 1629 cm.$^{-1}$. The compound is insoluble in ether but readily dissolves in chloroform to a red solution from which it can be extracted by dilute hydrochloric acid (6%) or aqueous resorcinol (50%). In a partition between aqueous methanol (50%) and carbon disulphide, the distribution was equal in each phase. As with erythroaphin, alkaline solutions are green and the sodium salt is insoluble in water. Diaminoerythroaphin-*fb* is stable to hot pyridine, to cold concentrated sulphuric acid, and to saturated sodium hydrogen carbonate solution. Mild reduction in acid or alkali gives the quinol reversibly, but stronger reduction causes deamination (see below). On an ascending paper chromatogram the compound gave discrete spots at R_F 0·63, with acetic acid–10% hydrochloric acid (1 : 3) as solvent, and at R_F 0·85 with acetic acid–10% hydrochloric acid (1 : 1). The characteristic colour change with acetic anhydride and sulphuric acid (Blount, *J.*, 1936, 1034; Part II, *loc. cit.*) occurred very slowly with diaminoerythroaphin and was not observed for about 1 hour under the normal reaction conditions.

The hydrochloride of the amine crystallised from a solution of the free amine in hydrochloric acid (3N). When shaken with water and chloroform the salt dissociated and the free base was obtained in the chloroform layer.

Dihydroxyerythroaphin-fb.—(i) *From diaminoerythroaphin*-fb. Diaminoerythroaphin-*fb* (100 mg.) was dissolved in concentrated hydrochloric acid (1 c.c.), acetic acid (3 c.c.), and water (6 c.c.). More hydrochloric acid (20 c.c. of 10%) was added to the cooled solution which was kept at 0° while a cooled solution of sodium nitrite (280 mg.) in water (200 c.c.) was added. The progress of the reaction was again followed by partition between ether and hydrochloric acid (10%) and by this means it was found that the reaction was virtually complete after 10 minutes. Excess of nitrite was destroyed with ammonium sulphamate, and the pigment extracted into ether (200 c.c.). The ethereal extract was washed with hydrochloric acid (10%) and then tartaric acid (0·5%), filtered if necessary, and concentrated. On addition of ethanol, *dihydroxyerythroaphin*-fb (45 mg.) crystallised as red prismatic needles which when heated decomposed without melting.

For analysis the substance was twice recrystallised from acetone and dried for 4 hours at 100°/1 × 10^{-4} mm. (Found: C, 66·6; 66·6; H, 4·4, 4·2; N, 0·0. $C_{30}H_{22}O_{10}$ requires C, 66·4; H, 4·1%). Light absorption in chloroform containing 5% of ether: Max. at 595, 567, 526, 490,

453, 426, 333, 320, and 253 mμ; log ε 3·76, 4·23, 4·06, 3·73, 4·62, 4·46, 3·66, 3·62, and 4·58 respectively. The spectrum in strong acids (cf. erythroaphin-*sl*; Part III, *J.*, 1950, 485), only momentarily visible in 70% sulphuric acid because of instability, can be observed with solutions in 60% perchloric acid or in acetic acid saturated with boric acid. The infra-red spectrum of a mull in Nujol showed maxima at 699, 771, 833, 869, 935, 944, 973, 1013, 1052, 1063, 1070, 1085, 1114, 1167, 1205, 1224, 1250, 1294, 1307, 1481, 1513, 1555, 1580, 1629, and 3268 cm.$^{-1}$.

The compound is stable in hot pyridine but rapidly changes from red to green in cold 70% sulphuric acid. It is also decomposed when chloroform or ether solutions are shaken with phosphate buffers of pH 5—8, saturated sodium hydrogen carbonate solution, or even water. Mild reversible reduction can be achieved in acid or alkaline media but stronger reduction causes dehydroxylation and formation of erythroaphin-*fb* (see below). In a partition between aqueous methanol (90%) and carbon disulphide, some 85% is retained in the methanol layer and it can be completely extracted from chloroform by 33% aqueous resorcinol. As in the case of erythroaphin, acid or neutral solutions are red and alkaline solutions are green. The sodium or potassium salts can be crystallised from alcohol or water as deep green prisms. The characteristic phase of the Blount colour reaction is passed too rapidly for observation unless acetic anhydride–phosphoric acid is used; the colour is then more purplish than that given by erythroaphin.

(ii) *From erythroaphin*-fb. A solution of erythroaphin-*fb* (500 mg.) and powdered potassium permanganate (1·0 g.) in pyridine (75 c.c.) containing a few drops of water was heated on a water-bath for 2 hours. The precipitated manganese dioxide was separated and washed with hot pyridine, the combined pyridine solutions were poured on ice (250 g.) and concentrated hydrochloric acid (100 c.c.), and the red precipitate was extracted with chloroform (4 × 100 c.c.). The red chloroform extracts were washed with water and then shaken thoroughly with N-sodium carbonate to extract the acidic product. The green aqueous layer was quickly washed with fresh chloroform and acidified with hydrochloric acid (3N). The red precipitate was extracted with chloroform, and the extract washed, dried, evaporated to small bulk (10 c.c.), and cooled to 0°. The product then separated as small red needles (55·2 mg.) which were recrystallised from acetone (Found, in material dried at 115°/0·1 mm. for 9 hours: C, 66·7; H, 4·0%). The ultra-violet and infra-red spectra of the product were identical with those recorded for the product obtained by the preceding method.

Erythroaphin-fb.—(i) *From diaminoerythroaphin*-fb. Diaminoerythroaphin-*fb* (100 mg.) was stirred in acetic acid (10 c.c.) at 90° and zinc dust (2·5 g.) was added during 2 minutes. The mixture was separated from the zinc which was washed with hot acetic acid. The combined filtrate and washings were poured into chloroform (100 c.c.), and a stream of air was bubbled through the solution in order to re-oxidise the pigment. The chloroform solution was thoroughly washed with sulphuric acid (64%) and then shaken overnight with sodium hydrogen carbonate solution. The filtered and washed chloroform solution gave erythroaphin-*fb* (20 mg.) as red needles, preferably separated from the hot solution by concentration and then addition of hot ethanol. For analysis it was twice recrystallised from chloroform–ethanol (Found: C, 70·25; H, 4·55. Calc. for $C_{30}H_{22}O_8$: C, 70·6; H, 4·35%). The ultra-violet and infra-red spectra agreed with those for authentic erythroaphin-*fb* (Parts II and V, *locc. cit.*).

(ii) *From dihydroxyerythroaphin*-fb. Dihydroxyerythroaphin-*fb* (38·6 mg.) was stirred with acetic acid (4 c.c.) and dioxan (1 c.c.) in a bath at 75—80°. Zinc dust (1 g.) was added, and the mixture stirred for 1 minute, cooled, and diluted with 1 : 1 : 1 (vol.) acetic acid–ether–chloroform. The zinc dust was separated and washed with more of the same solvent mixture. The filtrate was diluted with water, and the dissolved pigment re-oxidised by shaking it in air. Acid was removed from the organic layer with sodium hydrogen carbonate solution, and the chloroform–ether solution was then shaken for a further 2½ hours with an equal volume of saturated aqueous sodium hydrogen carbonate. Any solid material was separated from the organic layer, the solvent removed, and the residue dissolved again in pure chloroform. Further purification was effected by extracting the chloroform thoroughly with sulphuric acid (64%) and then transferring the pigment out of chloroform into sulphuric acid (72%) and finally back into chloroform by dilution (Part III, *loc. cit.*) The chloroform solution was shaken overnight with saturated sodium hydrogen carbonate solution, washed, filtered, and concentrated, and the erythroaphin-*fb* (12 mg.) crystallised by addition of hot ethanol (Found, in material dried at 125°/10^{-2} mm.: C, 70·7; H, 4·35%). The ultra-violet and infra-red spectra were identical with those of erythroaphin-*fb*.

Hydroxyerythroaphin-fb.—Erythroaphin-*fb* (500 mg.) was added to cold acetic anhydride

(100 c.c.) containing perchloric acid (10 drops of 60%), and the mixture stirred at 0° for 6½ hours whereafter the characteristic visible spectral lines of erythroaphin were no longer visible and the solution was blue with a powerful red fluorescence. Excess of anhydride was decomposed by adding the mixture to cold saturated sodium acetate solution (400 c.c.) and stirring for 1 hour at 0°. The intermediate dihydro-hydroxyerythroaphin-*fb* penta-acetate was extracted with ether (250 c.c.), and the extract thoroughly washed with 1% hydrochloric acid and then with sodium hydrogen carbonate solution. The yellow ethereal solution was now shaken with methanolic sodium hydroxide (3 c.c. of 10% aqueous sodium hydroxide in 27 c.c. of methanol) for a few minutes to convert the product into the green sodium salt of hydroxyerythroaphin-*fb*. The mixture was shaken with water, the aqueous layer separated and acidified with hydrochloric acid, and the product extracted with chloroform (250 c.c.). The chloroform extract was purified by shaking it for 3 hours with saturated sodium hydrogen carbonate solution and then filtered, washed, and concentrated. Addition of hot ethanol caused crystallisation of *hydroxyerythroaphin*-fb (362 mg.) as red needles and a further quantity (40 mg.) was obtained by concentration of the mother-liquors. For analysis a sample was recrystallised from chloroform–ethanol and dried at 110°/2 × 10^{-4} mm. (Found: C, 68·45, 68·4; H, 4·25, 4·2. $C_{30}H_{22}O_9$ requires C, 68·4; H, 4·2%). Light absorption in chloroform containing 5% of ether: Max. at 592, 563, 523, 488, 450, 425, 334, 319, and 254 mμ; log ε 3·81, 4·21, 4·05, 3·73, 4·59, 4·46, 3·64, 3·61, and 4·57 respectively. The infra-red spectrum of a mull in Nujol showed maxima at 721, 741, 766, 787, 827, 840, 867, 887, 942, 974, 1001, 1070, 1079, 1093, 1166, 1209, 1250, 1292, 1477, 1511, 1587, 1631, and 3226 $cm.^{-1}$. Hydroxyerythroaphin-*fb* is more stable to hot pyridine than erythroaphin. It is slowly decomposed by cold concentrated sulphuric acid but chloroform solutions are stable to sodium hydrogen carbonate solution unlike those of the dihydroxy-compound. Reversible reduction can be achieved under mild acid or alkaline conditions but stronger reduction causes dehydroxylation. The compound can be extracted from chloroform by sulphuric acid (68%) or by aqueous resorcinol (50%). In a partition between aqueous methanol (90%) and carbon disulphide, about 20% is retained in the methanol layer. Hydroxyerythroaphin forms red solutions in acidic or neutral solvents and green solutions in alkali.

Erythroaphin-fb *from Hydroxyerythroaphin*-fb.—(i) Zinc dust (1 g.) was added to a solution of hydroxyerythroaphin-*fb* (100 mg.) in boiling acetic acid, and the mixture shaken for 1 minute on the steam-bath. Chloroform and ether were added and the acetic acid was shaken out with water. Sodium hydroxide (100 c.c. of N) was added to precipitate the erythroaphin-*fb* as its green sodium salt which was separated and dissolved in chloroform containing acetic acid. The solution was thoroughly washed with water, sodium hydrogen carbonate solution, and finally sulphuric acid (64%). The pigment was then purified as described in the preparation from dihydroxyerythroaphin-*fb* (above) (Found, in a sample dried at 125°/10^{-2} mm.: C, 70·45; H, 4·55%). The ultra-violet and infra-red spectra were identical with those of erythroaphin-*fb*.

(ii) A solution of hydroxyerythroaphin-*fb* (51 mg.) in aqueous ammonia (*d* 0·88; 1 c.c.) and water (25 c.c.) was heated to boiling and sodium dithionite added in small quantities until the solution became orange-red. More sodium dithionite (200 mg.) was then added, the solution was boiled and then cooled, and sufficient 10% aqueous sodium hydroxide added to form the sodium salt of erythroaphin-*fb* after aerial oxidation. The sodium salt was separated, washed with dilute sodium hydroxide solution, and treated as in the previous experiment, to yield erythroaphin-*fb* (20 mg.) as red needles (Found: C, 70·3; H, 4·2%). The ultra-violet and infra-red spectra were identical with those of erythroaphin-*fb*.

Dipiperidinoerythroaphin-fb.—Erythroaphin-*fb* (25 mg.) was dissolved as far as possible in piperidine (5 c.c.), and the dark-green solution kept at room temperature for 24 hours with exclusion of light. The solution was then slowly stirred into ice-cooled hydrochloric acid (50 c.c. of 3N), and the dark-red product extracted with chloroform. The combined chloroform extracts were washed and dried, and the solvent was removed under reduced pressure. The residue was dissolved in hot pyridine (3 c.c.) and to this was added hot methanol (3 c.c.) containing 20% (v/v) of 3N-hydrochloric acid. After a few minutes a crystalline product separated which was twice recrystallised from the same solvent and finally washed free from excess of base and hydrochloride with hot water. *Dipiperidinoerythroaphin*-fb (16 mg.) crystallised in dark red plates which charred but did not melt at 200° (Found, in a sample dried at 100°/10^{-3} mm. for 3 hours: C, 71·1; H, 5·6; N, 4·1. $C_{40}H_{40}O_8N_2$ requires C, 71·0; H, 5·95; N, 4·1%). Light absorption in chloroform: Max. at 260—263, 445—448, 531, 575, and 605 mμ; log ε 4·52, 4·34, 4·14, 4·30, and 3·84 respectively. A solution of dipiperidinoerythroaphin-*fb* in chloroform was unchanged on irradiation in bright sunlight. The compound was soluble in

sulphuric acid (37%), and in stronger sulphuric acid (70%) the spectrum changed to the typical oxonium salt type (Part III; *loc. cit.*).

*Bis*cyclo*hexylaminoerythroaphin*-fb.—Erythroaphin-*fb* (250 mg.) was dissolved in a mixture of pyridine (20 c.c.) and *cyclo*hexylamine (2 c.c.), and the solution kept at room temperature overnight. The mixture was treated as in the foregoing preparation, and the *product* crystallised from pyridine (10 c.c.) and methanol (15 c.c.) containing 20% (v/v) of 3N-hydrochloric acid; it formed dark red plates (203 mg.). For analysis it was recrystallised twice more from the same solvent, washed with hot water, and dried at 100°/10^{-3} mm. (Found: C, 71·2; H, 6·3; N, 4·0. $C_{42}H_{44}O_8N_2$ requires C, 71·55; H, 6·3; N, 4·0%). Light absorption in chloroform: Max. at 258, 335, 460, 532, 573, and 606 mμ; log ε 4·55, 3·68, 4·37, 4·11, 4·21, and 3·81 respectively. In general properties the compound was very similar to the piperidine analogue.

Erythroaphin-fb.—(i) *From dipiperidinoerythroaphin*-fb. Dipiperidinoerythroaphin-*fb* (182 mg.) was suspended in acetic acid (10 c.c.), and zinc dust (2 g.) added. The mixture was heated on the steam-bath for 5 minutes with constant shaking, then cooled, and the excess of zinc separated. Erythroaphin-*fb* was formed from its leuco-compound by aerial oxidation, and the solution was poured into water (200 c.c.) from which the product was extracted with chloroform (3 × 35 c.c.). The product was purified as described in the preparation from dihydroxyerythroaphin-*fb* (above), and the product (71 mg.) recrystallised from chloroform–ethanol (Found: C, 70·2; H, 4·4%). The ultra-violet absorption spectrum in chloroform was identical with that of erythroaphin-*fb*.

(ii) *From bis*cyclo*hexylaminoerythroaphin*-fb. A similar procedure with di*cyclo*hexylaminoerythroaphin-*fb* (105 mg.) gave erythroaphin-*fb* (22 mg.) (Found: C, 70·2; H, 4·3%). The identity of the product was confirmed by the ultra-violet absorption spectrum.

Dichloroerythroaphin-fb.—Erythroaphin-*fb* (100 mg.) in chloroform (30 c.c.) was shaken with chlorine water (3 × 25 c.c.). The excess of chlorine was removed with sodium thiosulphate solution, and the chloroform finally washed with water and dried. Concentration to small bulk followed by addition of hot ethanol caused crystallisation of the product as with erythroaphin itself. *Dichloroerythroaphin*-fb was obtained as fine reddish-brown needles with a green lustre (82 mg.) which were recrystallised twice from the same solvent mixture (Found, in a sample dried at 100°/10^{-3} mm. for 3 hours: C, 58·0; H, 3·0; Cl, 18·85. $C_{30}H_{20}Cl_2O_8,\frac{1}{2}CHCl_3$ requires C, 57·4; H, 3·2; Cl, 19·4%). Light absorption in chloroform: Max at 263, 345, 450—452, 525, and 566 mμ; log ε 4·80, 3·84, 4·62, 4·31, and 4·42 respectively; inflexion at 470—473 mμ (log ε 4·57). The product was soluble in chloroform, sparingly soluble in ether, insoluble in ethanol, and soluble in concentrated sulphuric acid to a pinkish-violet solution. It gave an insoluble green sodium salt and charred but did not melt at 250°.

Dibromoerythroaphin-fb.—Erythroaphin-*fb* (100 mg.) was freeze-dried from dioxan and suspended in acetic acid (5 c.c.). Bromine (0·2 c.c.) was added and the suspension stirred for 1 hour. The mixture was then poured into water (80 c.c.) and the precipitate separated and washed with water and alcohol. After drying under reduced pressure the product (125 mg.) was crystallised three times from chloroform–ethanol and for analysis was dried at 100°/10^{-3} mm. overnight (Found: C, 50·3; H, 2·6. $C_{30}H_{20}Br_2O_8,\frac{1}{2}CHCl_3$ requires C, 50·2; H, 2·8%). Consistent halogen analyses could not be obtained with this compound by the conventional Carius method but the following semimicro-method gave reproducible results: Dibromoerythroaphin-*fb* (104·6 mg.; dried overnight at 110°/10^{-3} mm.) was weighed into a small glass phial and placed inside a Parr bomb with sodium (500 mg.). The bomb was heated at 550° in a muffle furnace for 1 hour after which it was cooled and the contents were digested with ethanol (30 c.c.). The insoluble residue was separated and washed with distilled water and the combined filtrates were acidified with nitric acid (12N; "AnalaR"), and silver nitrate solution (3 c.c. of 2%) was added slowly with stirring. The solution was boiled for 1 minute and set aside for 1 hour before determination of the silver halide gravimetrically in the usual manner. The precipitate was dried at 140° for 30 minutes before weighing (Found: AgBr + AgCl, 86 mg.; $C_{30}H_{20}Br_2O_8,\frac{1}{2}CHCl_3$ requires AgBr + AgCl, 85 mg.). In another experiment, dibromoerythroaphin-*fb* (76·3 mg.) gave 62·5 mg. of mixed silver halides ($C_{30}H_{20}Br_2O_8,\frac{1}{2}CHCl_3$ requires AgBr + AgCl, 62·0 mg.). Erythrophin-*fb* itself gave no measurable amount of silver halide under these conditions.

Diacetyldibromoerythroaphin-fb.—Pyridine (5 c.c.) was cooled in ice–salt, and acetyl chloride (2 c.c.) added dropwise. To this was added a solution of dibromoerythroaphin-*fb* (217 mg.) in pyridine (150 c.c.), and the mixture shaken for 5 minutes before benzene (150 c.c.) was added and the whole poured on ice (200 g.). The benzene layer was separated, quickly washed with water, dried, and concentrated to small volume at reduced pressure. The product was preci-

pitated by the addition of light petroleum (b. p. 40—60°), separated, dried, and purified by chromatography on silica (2 × 11 cm.) in benzene with development by benzene–acetone (100 : 1). The main orange band was washed through the column, the solvent mostly removed, and the product (88 mg.) precipitated by the addition of light petroleum. Rechromatography as before gave only a single band and the product was crystallised by slow evaporation at room temperature from benzene–light petroleum (b. p. 40—60°), forming long orange-yellow needles [Found : C, 54·4; H, 3·1; Br (Carius), 21·25. $C_{30}H_{18}Br_2O_6(O{\cdot}CO{\cdot}CH_3)_2$ requires C, 54·4; H, 3·2; Br, 21·3%]. Light absorption in chloroform : Max. at 263, 331—332, 361, and 445—456 mμ; log ε 4·58, 4·28, 4·17, and 4·65 respectively. The acetyl compound was very soluble in benzene and was readily deacetylated by alkali or even in hot ethanol.

Erythroaphin-fb *from Dibromoerythroaphin*-fb.—The dibromo-compound (144 mg.) was suspended in glacial acetic acid (10 c.c.) and zinc dust (2 g.) added. The mixture was heated on the water-bath for 30 minutes (the reduction being conveniently followed by observation of the visible spectrum through a hand spectroscope) and then poured into water (200 c.c.). The product was purified as was the dihydroxyerythroaphin-*fb* reduction, and the erythroaphin-*fb* (16 mg.) crystallised from chloroform–ethanol as before (Found : C, 70·2; H, 4·5%). The ultra-violet absorption spectrum was identical with that of erythroaphin-*fb*.

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