11. *Colouring Matters of the Aphididæ. Part XVIII.*[1] *The Structure and Chemistry of the Erythroaphins.*

By D. W. Cameron, R. I. T. Cromartie, Y. K. Hamied, P. M. Scott, and Lord Todd.

New structures (VII and VIII; R = R′ = H), differing only in the configuration at one centre and fully consistent with previous data, have been assigned to erythroaphin-*fb* and -*sl*, respectively. These compounds undergo dihalogenation by direct substitution of hydrogen in the perylene nucleus, while their characteristic diamination reactions follow a hitherto unknown pathway, involving attack at non-aromatic carbon atoms adjacent to the nucleus, in the course of which the stereochemical difference between the *fb* and the *sl* series is eliminated. From this follows the structure of dihydroxyerythroaphin-*fb* and from this, in turn, can be deduced the structure of the chemically related monohydroxy-compounds obtained on Thiele acetoxylation of erythroaphin-*fb*. Amination of dihalogenoerythroaphins in the presence of air leads to diaminated dihalogeno-derivatives; in its absence a novel displacement of halogen is observed. Some new reactions of substituted erythroaphins are discussed.

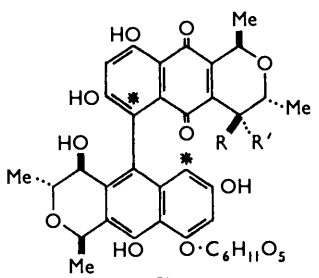
In Part XVII of this series [1] it was shown that protoaphin-*fb* and -*sl* are represented, both in general structure and absolute stereochemistry by formulæ (I; R = OH, R′ = H) and (I; R = H, R′ = OH), respectively. It is convenient now to discuss the isomeric erythroaphins-*fb* and -*sl* ($C_{30}H_{22}O_8$) which are the end-products of the remarkable series of interconversions characteristic of the aphin pigments of the *fb* and *sl* series. Because of their relative stability the erythroaphins have been investigated in considerable detail and a number of complex transformations which they undergo have already been reported.[2,3] In the present paper these earlier findings, amplified and in some instances modified by further work, will be re-interpreted in terms of new structures which will supersede the earlier tentative formulæ shown in Part XVI [4] to be inconsistent with later observations.

Early studies indicated that the erythroaphins were probably derivatives of 4,9-dihydroxyperylene-3,10-quinone (II), and this was confirmed beyond reasonable doubt by spectral comparisons of that quinone (II) and erythroaphin derivatives.[5] It remained to determine the nature of the non-aromatic portion of the erythroaphin molecule. This contains no reactive functional group and its structural elucidation was made additionally difficult by the fact that no degradation product containing more than two carbon atoms could be obtained from it. Four of the ten non-aromatic carbons were probably to be accounted for as *C*-methyl groups (Kuhn–Roth), and the four oxygen atoms not included in the central chromophore were evidently ethereal on the grounds of chemical inertness. It was also evident that this part of the molecule consisted of two similar groupings disposed on either side of the nucleus (II) in such a way as to make the erythroaphin molecule as a whole symmetrical. This was strongly supported by the simplicity of the nuclear magnetic resonance (n.m.r.) spectra of erythroaphin-*fb* derivatives which was consistent only with the exact superposition of two sets of bands representing identical groups of protons on either side of the central nucleus. Erythroaphin-*fb* could, therefore, be regarded as made up of a central chromophore (II) flanked symmetrically by two groups of five carbon atoms (two in *C*-methyl groups) and two ethereal oxygen

[1] Part XVII, Cameron, Cromartie, Kingston, and Todd, preceding paper.
[2] Brown, Johnson, MacDonald, Quayle, and Todd, *J.*, 1952, 4928.
[3] Brown, Calderbank, Johnson, MacDonald, Quayle, and Todd, *J.*, 1955, 954.
[4] Part XVI, Cameron, Cromartie, and Todd, *J.*, 1964, 48.
[5] Calderbank, Johnson, and Todd, *J.*, 1954, 1285.

atoms. Since erythroaphin-*sl* differs only stereochemically from the *fb* isomer similar structural conclusions apply to it also.

Resolution of the structural problem has been materially assisted both by the application of n.m.r. spectroscopy, which will be discussed in full elsewhere,[6] and by the work described in Part XVII [1] which related the protoaphins to naphthalene. In that paper it was shown that the two dithionite reduction products of the protoaphins had structures (III) or (IV), and (V) or (VI), and it was argued that, of all the structures possible for protoaphin on this basis, only (I) was compatible with the formation from them of the erythroaphins and with the chemistry of the latter. It is now necessary to amplify this argument and in so doing we shall arrive at structure (VII; R = R′ = H) for erythroaphin-*fb* and (VIII; R = R′ = H) for erythroaphin-*sl*. Spectral considerations indicate clearly that the protoaphins are 1,1′-binaphthyl derivatives. Based on the reduction products (III)—(VI), only two types of hydroxylated 1,1′-binaphthyl systems can be constructed, *viz.*, that shown in (I) and its analogue in which the linkage between the ring systems joins the two carbon atoms marked by asterisks in (I). Either of these structures can, in principle, give rise to the erythroaphin chromophore (II) effectively by condensation of one of the naphthaquinone-carbonyl groups on an aromatic carbon appropriately activated towards electrophilic attack by phenolic hydroxyl (the actual process will be discussed in a subsequent paper [7]), but only formula (I) can yield in this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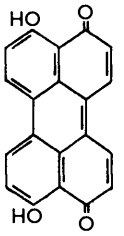
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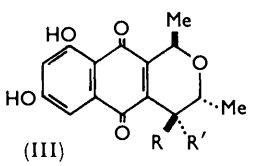
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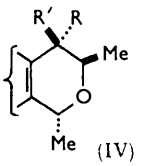
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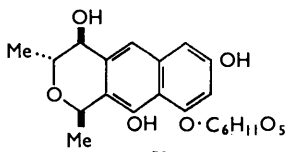
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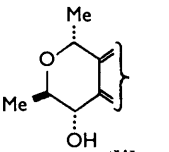
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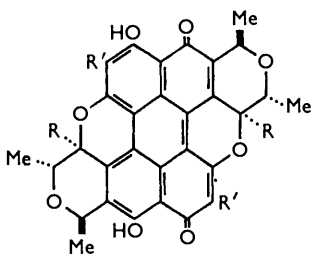
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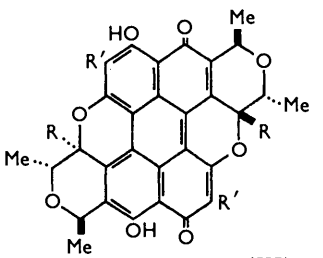
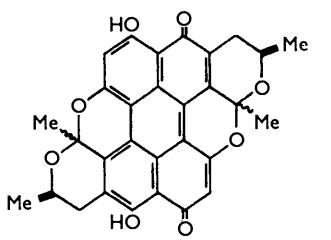

(VIII)


(IX)

way a product capable of giving a coronene derivative on zinc dust fusion as is observed with the erythroaphins.[8] This consideration establishes the protoaphin chromophore, but it does not distinguish between the alternative side groupings exemplified by (III) and (V) on the one hand and (IV) and (VI) on the other. This distinction can be made, however, on the basis that in formula (I; R, R′ = H, OH) two pairs of alcoholic and nearby phenolic hydroxyl groups (all of which are absent in the erythroaphins) are suitably placed for simple dehydration so as to yield the two additional ether groupings required

[6] Part XXI, Cameron, Cromartie, Hamied, Scott, Sheppard, and Todd, *J.*, 1964, 90.
[7] Part XX, Calderbank, Cameron, Cromartie, Hamied, Haslam, Kingston, Todd, and Watkins, *J.*, 1964, 80.
[8] Brown, Johnson, Quayle, and Todd, *J.*, 1954, 107.

in any erythroaphin structure. In the alternative type of structure the hydroxyl groups are remote from one another, direct dehydration cannot occur, and, although reaction schemes can be devised leading to (IX), the chemical properties of which might have a general resemblance to those of erythroaphin, such a structure is quite incompatible with the results of n.m.r. spectroscopic studies, in that, among other discrepancies, it contains two quaternary methyl groups and two methylene groups attached directly to the chromophore.[6] On this basis, therefore, erythroaphin-*fb* is formulated as (VII; R = R′ = H) and erythroaphin-*sl* as (VIII; R = R′ = H) which differ only in the configuration at one centre (although there may be a considerable difference in conformation). In arriving at these structures the assumption has, of course, been made that in the change protoaphin ⟶ erythroaphin no rearrangement of the non-aromatic portions occurs, but this assumption is warranted by the marked similarity in the n.m.r. spectra of protoaphin and erythroaphin derivatives.

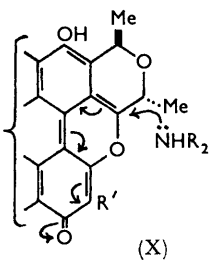
Structures (VII) and (VIII) meet the objections raised in Part XVI [4] to the structures proposed earlier. They contain only two CH groups in the chromophore, these being the points at which halogenation occurs. The process of halogenation does not involve the centre which is stereochemically different in the two erythroaphins and the existence of distinct halogeno-compounds in the *fb* and *sl* series is readily understood. These formulæ are well supported by the results of n.m.r. spectroscopic studies and in suitable derivatives it is possible to make detailed assignments of position to every proton in the molecule. Furthermore, the presence of two ethereal oxygens directly linked to the chromophore explains the observed bathochromic shift in the electronic absorption of tetra-acetyldihydroerythroaphins relative to that of 3,4,9,10-tetra-acetoxyperylene.[5]

We may now consider the general problem of the amination of erythroaphins. It has already been shown that treatment of erythroaphins-*fb* and -*sl* (VII and VIII; R = R′ = H) with ammonia or primary or secondary amines under mild conditions yields, in each case, one and the same diaminated erythroaphin and that similar treatment of dibromoerythroaphins-*fb* and -*sl* (VII and VIII; R = H, R′ = Br) yields one diaminodibromoderivative.[2,3] These aminated compounds have been assigned to the *fb*-series since they are readily reduced to erythroaphin-*fb*. (Reduction is conveniently brought about by zinc and acetic acid but it can also be effected catalytically, *e.g.*, hydrogenation of dibromodipiperidinoerythroaphin-*fb* proceeds with the uptake of 5·1 mol. of hydrogen, four mol. being required for removal of the four substituents and the fifth for reduction of the quinone.) The *sl* ⟶ *fb* epimerisation on amination is apparently quantitative and is consistent with thermodynamic considerations, since erythroaphin-*sl* can be converted into the *fb*-isomer simply by treatment with alkali.[9] The processes described above are strongly reminiscent of the well-known amination of simple quinones, two general types of which are known. The first involves nucleophilic addition of amine to an unsubstituted quinone, yielding a monoamino-quinol, followed by oxidation to the corresponding amino-quinone, and repetition of the process in the introduction of a second amino-group. This type is characterised by the oxidation steps it includes. These in the present case would be atmospheric, because of the great ease with which erythroaphin quinols undergo re-oxidation to the quinones and would be manifested in theory by an uptake of 1 mol. of oxygen for each amine group introduced. However, such a route could accommodate the ready formation of diaminodibromoerythroaphins only if the unsubstituted erythroaphins contained four unsubstituted " nuclear " positions, a situation which has already been excluded on other grounds. The second general type of quinone amination involves simple displacement of other nucleophiles, *e.g.*, OR, and this also occurs under very mild conditions. No oxygen uptake would be expected in such displacements (this was verified by the simple model conversion of 2,5-dichloro-3,6-dimethoxybenzoquinone into the corresponding 3,6-bisbutylamino-derivative). Such a mechanism

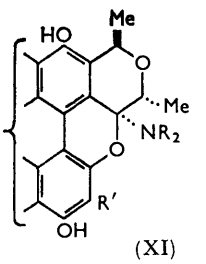
[9] Johnson, Todd, and Watkins, *J.*, 1956, 4091.

for the erythroaphins (VII, VIII) is most unlikely, however, because the only groups potentially able to undergo displacement in this way are the ether-oxygens in "waist" positions of the perylene system. The steric problems in accommodating groups as bulky as piperidine, in those positions, when the remainder of the nucleus is so heavily substituted, are very great. In any event, reaction of erythroaphin-*fb* (VII; R, R′ = H) and dibromoerythroaphin-*sl* (VIII; R = H, R′ = Br) with piperidine proceeded with an uptake of 2·2 and 1·5 mol. of oxygen, respectively. (Theory for oxidative diamination is 2·0 mol.; the lower uptake in the latter case will be discussed later.) Similar treatment of dibromoerythroaphin-*fb* (VII; R = H, R′ = Br) with triethylamine caused no absorption of oxygen and starting material was recovered in good yield. This indicates that the oxygen uptakes recorded above are not due to the formation of by-products formed by oxidation under the basic conditions, but are significant for the reactions under investigation. In this connexion we have used the technique of oxygen-uptake measurement extensively and found results to be reproducible, although for less heavily substituted quinones, where a greater possibility of oxidative side-reactions exists, it might have been of less value.

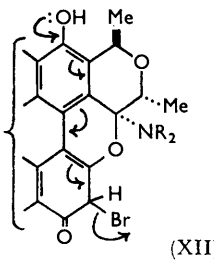
The structures of diamino- and diaminodibromo-erythroaphin were shown to be (VII; R = NR_2, R′ = H; and R = NR_2, R′ = Br, respectively) by n.m.r. spectroscopy (detailed discussion is deferred to a subsequent paper[6]). The spectrum of dipiperidinoerythroaphin-*fb* (VII; R = piperidino, R′ = H) contained three features of significance, as compared with those of non-aminated derivatives: (*a*) the "aromatic" protons were still present and of undiminished intensity; (*b*) the doublet due to protons R in (VII), coupled with the adjacent protons, was no longer present; and (*c*) the same adjacent protons which in non-aminated derivatives are present as an unsymmetrical pair of quartets were in the dipiperidino-compound reduced to a single quartet. No other formulation than (VII; R = piperidino, R′ = H) is consistent with these facts. Although it is apparently a crowded system when written two-dimensionally, models of it can be constructed easily. We have examined the spectra of a large number of related derivatives and on this basis make the generalisation that amination proceeds exclusively at positions R in (VII) and halogenation at R′. The mechanism of the amination involving, as it does, alkylated quinones, merits closer attention, because a reaction of this nature does not appear to have been observed before. We have shown it to apply with equal ease to a number of simpler alkylated quinones, *e.g.*, duroquinone, and it appears to be general.[10] In the erythroaphin case it can be envisaged as involving a reactive tautomer as in the partial formula (X; R′ = H). Nucleophilic addition gives rise to the amino-quinol (XI; R′ = H) which absorbs 1 mol. of oxygen, yielding the amino-quinone and so on. The epimerisation



(X)



(XI)



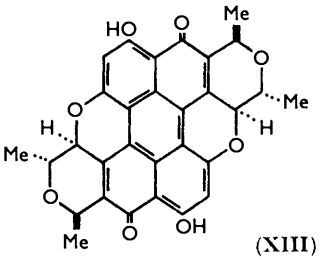
(XII)

sl ⟶ *fb* which invariably accompanies the process is now readily explained by the fact that in the intermediate (X) the stereochemical difference between the two isomers is obliterated. The reaction as a whole is analogous to the reaction of unsubstituted quinones leading to nuclear amination, and the properties of diaminoerythroaphins resemble more closely those of aromatic than of aliphatic amines, a fact which confused earlier structural work. For example, they have apparently low basicity and their visible

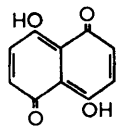
[10] Cameron, Scott, and Todd, *J.*, 1964, 42.

spectra are bathochromically shifted up to 15 mμ relative to their non-aminated analogues. However, analogous spectral changes have also been observed in the aminated derivatives of the simpler quinones mentioned above, whose structures have been confirmed by synthesis.[10] Such effects might be explained in the erythroaphin case by direct interaction between the lone-pair nitrogen electrons and the π-system of the chromophore; an interaction of this nature appears feasible on the basis of molecular models. The ease with which the substituents in aminoerythroaphins can be removed reductively,[2,3] which would also have been consistent with nuclear amination, is now explained by the fact that the amino-groups in the leuco-compounds are on benzyl carbons which are also adjacent to ethereal oxygen atoms.

In order to understand satisfactorily the aminations and halogenations that we have discussed so far, it is necessary to consider the erythroaphin chromophore in (VII) as only one of a number of possible tautomeric forms, obtained by movement of the phenolic protons between *peri*-oxygen atoms. It would be expected that any one of the possible mobile tautomers could be involved as reaction intermediates should this be necessary. For example, to explain *di*amination on the basis of (VII) it is necessary to envisage first one side of the molecule and then the other as " quinonoid " rather than " benzenoid." On this basis it is interesting that amination does not appear to occur to any extent so as to give a compound (VII; R = H, R′ = NR_2), and a possible conclusion is that the relevant positions in the molecule are, in fact, " benzenoid," at least under these relatively mild reaction conditions. If this is so, a more satisfactory structure for erythroaphin-*fb* might be (XIII) which is completely symmetrical. In general, simple perylene-3,10-quinones seem to be favoured over their 3,9-isomers. For example, direct oxidation of perylene gives the 3,10-quinone,[11] while the diacetylerythroaphins are, from their spectra, unquestionably the 3,10-derivatives.[12] Despite this it does not follow that (VII) is necessarily preferable to (XIII) for erythroaphin itself; the question can be solved only by physical methods and in this connexion it is interesting that recent crystallographic studies of naphthazarin suggest that it may exist in the form (XIV).[13]



(XIII)



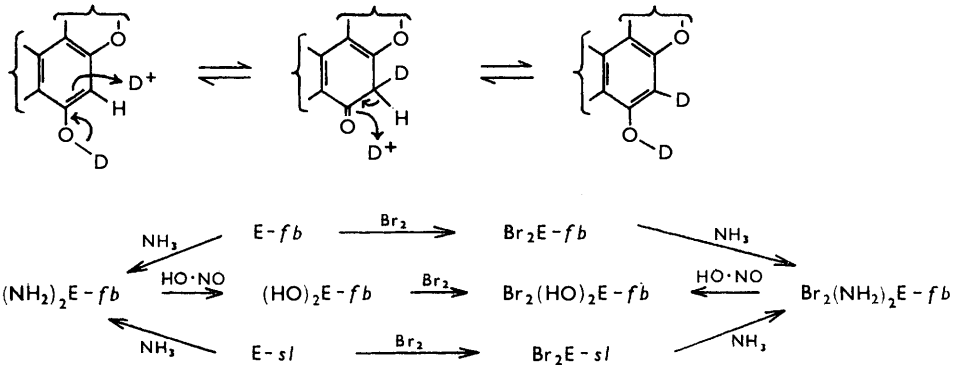
(XIV)

When erythroaphin-*fb* was treated with piperidine in the absence of oxygen, a mixture of dipiperidinoerythroaphin-*fb* and unchanged starting material was obtained. This was not unexpected since in the absence of air the intermediate amino-quinol (XI) would be oxidised at the expense of unchanged erythroaphin, which then would remain in the reduced form until the reaction was worked up. However, similar treatment of dibromoerythroaphin-*fb* or -*sl* (VII, VIII; R = H, R′ = Br) with piperidine gave rise, in good yield, to dipiperidinoerythroaphin-*fb* (VII; R = piperidino, R′ = H). This striking reaction probably occurs by tautomeric change of the amino-quinol intermediate (XI; R′ = Br) to the keto-form (XII) followed, as shown, by elimination of hydrogen bromide under the basic conditions in which the reaction is carried out. This leads to the amino-quinone without an oxidation step. Such a process may well explain the ready reductive

[11] Elsevier's " Encyclopedia of Organic Chemistry," Elsevier, New York, 1940, Series 3, Vol. XIV, p. 467.
[12] Human, Johnson, MacDonald, and Todd, *J.*, 1950, 477.
[13] Cf. Pascard-Billy, *Acta Cryst.*, 1962, **15**, 519.

removal of bromo-substituents from the erythroaphin nucleus. Tautomerism of the type (XI) ⇌ (XII) is observed in the reduced forms of other hydroxylated quinone systems [14] and is strongly supported in the present case by the deuteration experiments described below. Since anaerobic amination proceeds at a rate comparable with that of oxidative amination it might be expected to compete with it in air, *i.e.*, the intermediate (XI; R′ = Br) can either be oxidised to the corresponding quinone or eliminate hydrogen bromide *via* (XII). We believe that this explains the low uptake (1·5 mol. of oxygen) actually observed in this reaction (see above). Similar amination of the dichloroerythroaphins proceeds with absorption of 1·9 mol. of oxygen, which is consistent with the expected slower elimination of hydrogen chloride. On the other hand, treatment of di-iodoerythroaphins yields as the only isolable product the *mono*iododipiperidino-derivative (which can be converted reductively into erythroaphin-*fb*). This illustrates the ease with which anaerobic amination occurs in this case. The di-iodo-derivatives used were prepared by treatment of the respective erythroaphins, into which they can be reconverted reductively, with iodine monochloride.

The structures we have assigned to a number of substituted erythroaphins are supported by the products they yield on reduction with zinc and deuterioacetic acid ($CH_3 \cdot CO_2D$). This reagent reduces almost all amino- and halogeno-derivatives back to the parent erythroaphin, except those containing chloro-substituents which are stable under these conditions.[15] When erythroaphin-*fb* is treated in this way and the leuco-compound allowed to reoxidise, the product contains an aromatic C–D band in the infrared spectrum at 2285 cm.$^{-1}$ and is formulated as the dideuterioerythroaphin (VII; R = H, R′ = D). Incorporation of isotope into the leuco-compound probably occurs by the scheme indicated below, providing further evidence for the tautomerism (XI) ⇌ (XII). Refluxing erythroaphin with deuterioacetic acid alone results in no incorporation. As expected, similar treatment of dichloroerythroaphin-*sl* yielded unchanged material which contained no deuterium, whereas the dibromo-analogue yielded the same dideuterio-derivative (VII; R = H, R′ = D) as was obtained from erythroaphin itself. (This assignment was confirmed by n.m.r. spectra which showed the absence of aromatic CH.) Dipiperidino- and dibromodipiperidino-erythroaphin-*fb* yielded the same new product, formulated as the tetradeuterio-derivative (VII; R = R′ = D). In addition to having a band at 2285 cm.$^{-1}$ this compound also showed another at *ca.* 2150 cm.$^{-1}$, due to the non-aromatic CD group.[16] The latter band was weaker than the former, as might have been expected,[17] and the differences brought about in the fingerprint infrared region by the new pair of deuterium atoms, were a more significant criterion for their presence.



[14] Thomson, *Quart. Rev.*, 1956, **10**, 27.
[15] Cameron, Cromartie, Hamied, Joshi, Scott, and Todd, following paper.
[16] Alexander and Pinkus, *J. Amer. Chem. Soc.*, 1949, **71**, 1786.
[17] Bellamy, " The Infra-red Spectra of Complex Molecules," Methuen, London, 1959, p. 19.

Hitherto, " tetra-substituted " erythroaphins [(VII) in which R and R′ are introduced substituents] have been prepared only by amination of dihalogeno-derivatives followed by subsequent transformation if necessary.[3] The reverse potential method of formation, *viz.*, halogenation of a diamino- or related derivative has never been achieved and we have confirmed earlier observations [3] that bromination of dipiperidino- or diamino-erythroaphin-*fb* (VII; R = NR_2, R′ = H) leads to non-basic products. Indeed, the expected products dibromodipiperidino- and diaminodibromo-erythroaphin-*fb* (VII; R = NR_2, R′ = Br) are themselves attacked by halogen in a similar way, reaction presumably being initiated by electrophilic attack on the amino-nitrogen atom. However, dihydroxyerythroaphin-*fb*, which, being formed by the action of nitrous acid on its diamino-analogue, is now formulated as (VII; R = OH, R′ = H), reacts smoothly with bromine, yielding dibromodihydroxyerythroaphin-*fb* (VII; R = OH, R′ = Br). This has also been obtained by diazotisation of the diaminodibromo-compound as reported previously. This cycle of reactions is illustrated in the annexed chart (E = erythroaphin). A similar bromination of diacetamidoerythroaphin-*fb* [15] (VII; R = NHAc, R′ = H) yields the diacetamidodibromo-derivative (VII; R = NHAc, R′ = Br), and the halogenation thus appears to be general provided the R substituent in (VII) is not basic.

Thiele acetoxylation of the erythroaphins, as reported earlier, yields after hydrolysis monohydroxy-derivatives.[2] This reaction is, in general, mechanistically analogous to the amination of quinones except that the reactive species is the protonated rather than the free quinone, the reaction being carried out in the presence of strong acid. Despite this difference and the fact that simpler alkylated quinones, *e.g.*, duroquinone, are resistant under these conditions, in contrast to their ready reaction with amines, hydroxyerythroaphin-*fb* is formulated as (VII; R′ = H, in which one of the groups R is OH). This has been established as follows. Treatment of the monohydroxy-compound with piperidine involves absorption of 1·0 mol. of oxygen and formation of the previously reported monohydroxymonopiperidino-derivative. This reacted with acetyl chloride in pyridine to yield diacetyldihydroxyerythroaphin-*fb*,[15] which was not isolated but which on hydrolysis was converted into dihydroxyerythroaphin-*fb*, identical with the material already formulated as (VII; R = OH, R′ = H). In addition, bromination of hydroxyerythroaphin-*fb* yields what is probably a dibromo-derivative, whose presence was also detected paper chromatographically on Thiele acetoxylation of dibromoerythroaphin-*fb*, though it could not be isolated in a pure state from the latter reaction.

On the basis of structure (VIII; R = R′ = H) Thiele acetoxylation of erythroaphin-*sl* (as distinct from the *fb* isomer) could in theory yield two distinct products according to which side of the molecule were attacked, and hydrolysis of these would give hydroxyerythroaphin-*sl* and -*fb*, respectively. This undoubtedly explains the curious result recorded in Part XIV [18] where it was reported that, although " hydroxyerythroaphin-*sl* " appeared to differ slightly from hydroxyerythroaphin-*fb* in infrared spectrum, it nevertheless gave erythroaphin-*fb* (albeit in poor yield) on treatment with zinc dust and acetic acid. Re-examination of this matter shows that the product obtained by Thiele acetoxylation of erythioaphin-*sl* followed by hydrolysis is not homogeneous. Paper chromatography shows it to consist of two very similar components, one of which is hydroxyerythroaphin-*fb*; isolation of the second component (presumably hydroxyerythroaphin-*sl*) in a pure condition has not been attempted.

It was reported earlier [19] that, when heated with zinc chloride or with 62% sulphuric acid, the erythroaphins yield acetaldehyde. The production of acetaldehyde under these conditions is, of course, compatible with structures (VII) and (VIII), but the claim that erythroaphin-*fb* yielded twice as much acetaldehyde on acid treatment as did erythroaphin-*sl* was difficult to reconcile with the new formulæ. We have accordingly re-examined

[18] Brown, Calderbank, Johnson, Quayle, and Todd, *J.*, 1955, 1144.
[19] Brown, Calderbank, Johnson, Joshi, Quayle, and Todd, *J.*, 1955, 959.

the reaction between the erythroaphins and 62% sulphuric acid under various conditions. The yields of acetaldehyde (isolated as its 2,4-dinitrophenylhydrazone) obtained are very variable ranging from 0·4 to 1·6 mol. and there is no significant difference between erythroaphin-*fb* and -*sl* in this respect. Earlier apparent differences were probably due to differences in physical form of the aphins and to the speed of the nitrogen stream employed to carry away the acetaldehyde—both of them factors which we have observed to give variations in yield in our re-examination of the problem. Similar results were obtained on acid treatment of the dihydro-*O*-tetramethylerythroaphins-*fb* and -*sl*[15]—variable yields of acetaldehyde up to 1·6 mol. with no difference between the isomers. The apparent difficulty of accommodating earlier results on acetaldehyde production with the new erythroaphin structure is thus removed.

Experimental

Unless otherwise stated, ultraviolet and visible spectra were measured for chloroform solutions and infrared for Nujol and/or hexachlorobutadiene mulls. Measurements of oxygen uptake were made volumetrically in a micro-hydrogenation apparatus filled with carbon dioxide-free air, light being excluded as far as possible by enclosing the reaction flasks with aluminium foil. Anaerobic experiments were carried out in the same apparatus filled with carbon dioxide-free and oxygen-free nitrogen.

Aminations in Air.—(*a*) 2,5-Dichloro-3,6-dimethoxybenzoquinone (33·2 mg.) in benzene (2 ml.) was set aside with n-butylamine (20 drops). No oxygen uptake was observed during the reaction which appeared complete after 30 min. The product, 2,5-bisbutylamino-3,6-dichlorobenzoquinone, was recrystallised twice from dioxan and formed pink needles (29 mg.), m. p. 198—200° (lit.,[20] m. p. 202—203°).

(*b*) Erythroaphin-*fb* (51·0 mg.) was treated with piperidine (4 ml.) at room temperature, to give dipiperidinoerythroaphin-*fb* (41·0 mg.).[2] Reaction was complete after 24 hr. (oxygen uptake, 2·2 mol.). Dipiperidinoerythroaphin-*fb* as a blank absorbed no oxygen under these conditions.

(*c*) Dibromoerythroaphin-*sl* (100·2 mg.) in piperidine (6 ml.) gave the dibromodipiperidino-*fb* derivative[3] (50 mg.), absorbing 1·5 mol. of oxygen in 19 hr. Dibromoerythroaphin-*fb* behaved similarly. It was necessary to exclude light during these determinations as a solution of dibromodipiperidinoerythroaphin in piperidine took up oxygen in daylight, but not in the dark.

(*d*) Dibromoerythroaphin-*fb* (68·8 mg.) was treated with triethylamine (4 ml.) as above. No oxygen uptake occurred during 2·5 hr.; then the mixture was acidified and extracted with chloroform, and material from the extract was crystallised from chloroform–ethanol, to yield starting material (55 mg.), identified by its infrared spectrum.

(*e*) Dichloroerythroaphin-*fb* (63·9 mg.) and piperidine (4 ml.) reacted as above with an uptake of 1·9 mol. of oxygen, complete after 50 hr.[15]

Anaerobic Aminations.—(*a*) Erythroaphin-*fb* (106 mg.) was treated at room temperature with piperidine (4 ml.) under nitrogen. After 22 hr. the mixture had acquired a dark orange colour which rapidly became green on exposure to air. The mixture was shaken with chloroform (50 ml.) and 3N-hydrochloric acid (25 ml.) at 0°. The red chloroform layer was shaken with concentrated hydrochloric acid (3 × 20 ml.), washed, and dried. Evaporation of solvent and crystallisation from chloroform–ethanol gave erythroaphin-*fb* (43 mg.), identified spectroscopically. The acid extracts were immediately diluted with water (50 ml.) and extracted with chloroform (4 × 15 ml.); these chloroform extracts were washed, dried, and evaporated. The residue, on crystallisation from chloroform–ethanol, gave dipiperidinoerythroaphin-*fb* (20·5 mg.), identified spectroscopically.

(*b*) Dibromoerythroaphin-*sl* (113 mg.) and piperidine (7 ml.) were allowed to react as above. The mixture was acidified at 0°, extracted into chloroform and then concentrated hydrochloric acid (3 × 20 ml.) as above. Immediate dilution of the acid extract with water (150 ml.) at 0° and re-extration with chloroform (3 × 20 ml.) gave, after washing, drying, and evaporation, dipiperidinoerythroaphin-*fb* (77 mg.) which was purified as above and crystallised from pyridine–80% aqueous methanol. Its ultraviolet and infrared spectra were identical with

[20] Buckley, Henbest, and Slade, *J.*, 1957, 4891.

those of authentic material (Found: C, 70·9; H, 6·4; N, 4·2. Calc. for $C_{40}H_{40}N_2O_8$: C, 71·0; H, 6·0; N, 4·1%).

(*c*) Dibromoerythroaphin-*sl* (147 mg.) and piperidine (8 ml.), treated as above, also yielded dipiperidinoerythroaphin-*fb* (84 mg.), identified spectroscopically.

(*d*) The same product (29 mg.) was obtained when dichloroerythroaphin-*fb* (50 mg.) and piperidine (4 ml.) were left under nitrogen at room temperature for 24 hr.

Dichloroerythroaphin-sl.—Erythroaphin-*sl* (150 mg.) was chlorinated as for the *fb* isomer,[2] to yield *dichloroerythroaphin*-sl (85 mg.), forming red needles on recrystallisation from chloroform–ethanol (Found: C, 62·0; H, 3·6; Cl, 12·5. $C_{30}H_{20}Cl_2O_8$ requires C, 62·2; H, 3·5; Cl, 12·3%); $\lambda_{max.}$ 261, 347, 456, 522, and 563 mμ (log ε 4·61, 3·63, 4·45, 4·13, and 4·26); $\nu_{max.}$ 1626, 1567, 1350, 1326, 1300, 1250, 1201, 1167, 1140, 1110, 1080, 1048, 1004, 977, 967, 912, 856, 835, 824, 808, 775, and 750 cm.$^{-1}$. Dichloroerythrophin-*fb* [2] has infrared absorption at 1624, 1568, 1304, 1261, 1239, 1203, 1155, 1108, 1075, 1050, 1000, 960, 895, 853, 825, 800, and 750 cm.$^{-1}$.

Di-iodoerythroaphin-sl.—A suspension of erythroaphin-*sl* (210 mg.) in glacial acetic acid (20 ml.) and iodine monochloride (200 mg.) was shaken at room temperature for 1·5 hr. The mixture was poured into water (200 ml.), and the precipitated product centrifuged, washed with water, aqueous sodium thiosulphate, and again with water, dried, and dissolved in chloroform (200 ml.). The chloroform solution was washed with aqueous sodium thiosulphate, water, aqueous sodium hydrogen carbonate, and water, dried, and evaporated. Crystallisation of the resulting gum from chloroform containing a trace of ethanol gave *di-iodoerythroaphin*-sl (145 mg.) as deep red-brown needles (Found: C, 46·9; H, 2·7; I, 33·8. $C_{30}H_{20}I_2O_8$ requires C, 47·3; H, 2·6; I, 33·4%), $\lambda_{max.}$ 267, 337, 453, 486, 527, and 569 mμ (log ε 4·64, 3·68, 4·40, 4·36, 4·22, and 4·30), $\nu_{max.}$ 1616, 1574, 1304, 1263, 1212, 1195, 1160, 1116, 1075, 1045, 1000, 972, 955, 852, 836, 805, and 765 cm.$^{-1}$.

Treatment of this compound (20 mg.) with zinc and acetic acid in the usual way [2] yielded erythroaphin-*sl* (8 mg.), identified spectroscopically.

Di-iodoerythroaphin-fb.—Erythroaphin-*fb* (220 mg.), iodinated as above, gave after two recrystallisations from chloroform–ethanol *di-iodoerythroaphin*-fb (210 mg.) as deep red-brown needles (Found: C, 47·4; H, 2·9; I, 33·1%), $\lambda_{max.}$ 268, 337, 453, 488, 526, and 569 mμ (log ε, 4·64, 3·68, 4·38, 4·37, 4·23, and 4·30), $\nu_{max.}$ 616, 1592, 1302, 1257, 1213, 1198, 1155, 1100, 1075, 1045, 998, 952, 934, 890, 852, 835, 825, 801, and 745 cm.$^{-1}$.

Treatment of this compound (15 mg.) with zinc and acetic acid in the usual way [2] gave erythroaphin-*fb* (5 mg.), identified spectroscopically.

Mono-iododipiperidinoerythroaphin-fb.—(*a*) Di-iodoerythroaphin-*fb* (200 mg.) was treated with piperidine in the usual way.[3] The product crystallised twice from chloroform–ethanol, to yield deep red *iododipiperidinoerythroaphin*-fb (90 mg.) (Found: C, 60·2; H, 5·0; I, 15·6; N, 3·5. $C_{40}H_{39}IN_2O_8$ requires C, 59·8; H, 4·9; I, 15·8; N, 3·5%), $\lambda_{max.}$ 264, 448, 537, and 579 mμ (log ε 4·57, 4·28, 4·24, and 4·31), $\lambda_{infl.}$ 470 mμ (log ε 4·23); $\nu_{max.}$ 1627, 1578, 1346, 1307, 1284, 1241, 1215, 1185, 1155, 1116, 1100, 1080, 1043, 1021, 967, 918, 908, 878, 855, 815, and 763 cm.$^{-1}$.

(*b*) Di-iodoerythroaphin-*sl* (175 mg.), aminated as above, yielded the same product (90 mg.), whose identity was confirmed by spectral comparisons.

Reduction of mono-iododipiperidinoerythroaphin-*fb* (20 mg.) in the usual way,[3] by zinc and acetic acid, yielded erythroaphin-*fb* (4 mg. after recrystallisation), whose identity was confirmed by spectral comparison.

Deuteration Experiments.—Deuterioacetic acid ($CH_3{\cdot}CO_2D$) was prepared by refluxing molar quantities of acetic anhydride and D_2O for 12 hr.[21]

(*a*) Erythroaphin-*fb* (70 mg.) was suspended in $CH_3{\cdot}CO_2D$ (10 ml.), and zinc dust (1 g.) was added. The mixture was heated on the water-bath for 40 min., then cooled and the excess of zinc separated. The filtrate was poured into water, and the product was extracted into chloroform (100 ml.) which was then washed, dried, and evaporated. The resultant red gum crystallised from chloroform–ethanol, yielding dideuterioerythroaphin-*fb* (VII; R = H, R′ = D) (37 mg.) as red needles. Its visible spectrum was identical with that of non-deuterated material. Infrared absorption was at 2284 (aromatic CD), 1627, 1580, 1303, 1263, 1243, 1203, 1181, 1158, 1126, 1107, 1086, 1047, 1007, 947, 896, 869, 828, 808, 777, and 763 cm.$^{-1}$.

[21] Weltner, *J. Amer. Chem. Soc.*, 1955, **77**, 3941.

(*b*) Reduction of dibromoerythroaphin-*fb* (120 mg.) as above gave dideuterioerythroaphin-*fb* (VII; R = H, R′ = D) (30 mg.) whose spectra were identical with those of the product from (*a*).

(*c*) Reduction of dichloroerythroaphin-*sl* (35 mg.) as above yielded starting material (16 mg.). Its identity was confirmed by spectral comparison.

(*d*) Dipiperidinoerythroaphin-*fb* (110 mg.) similarly yielded tetradeuterioerythroaphin-*fb* (VII; R = R′ = D) (40 mg.) that crystallised from chloroform–ethanol as red needles whose visible spectrum was identical with that of the erythroaphins but whose infrared absorption was at 2280 (aromatic CD), 2150 (v.w. aliphatic CD), 1627, 1580, 1290, 1245, 1165, 1138, 1125, 1106, 1084, 1052, 1031, 1010, 992, 963, 895, 867, 828, 806, 775, and 758 cm.$^{-1}$.

(*e*) Similarly, reduction of dibromodipiperidinoerythroaphin-*fb* (60 mg.) yielded the same product (20 mg.) as from (*d*), on the basis of spectra.

Attempted Bromination of Aminated Erythroaphins.—(*a*) Treatment of dipiperidinoerythroaphin-*fb* (6·8 mg.) in chloroform (1 ml.) with bromine (3·1 mg.) rapidly gave a mixture of products (separated on a paper chromatogram) which could not be extracted into dilute hydrochloric acid.

With acetic acid as solvent for bromination the product showed one spot only on paper chromatograms ($\lambda_{max.}$ 540 and 585 mμ) but it was again non-basic.

(*b*) Dibromodipiperidinoerythroaphin-*fb* (8·5 mg.) was treated as above with bromine (3·1 mg.) in chloroform. The visible spectrum rapidly changed, a band appearing at 590 mμ and paper chromatography after 10 min. revealed a number of unidentified products.

Dibromodihydroxyerythroaphin-fb.—(*a*) Diaminodibromoerythroaphin-*fb* (75 mg.) was diazotised in the usual way [2] and the resulting red gum crystallised twice from acetone–cyclohexane, to give *dibromodihydroxyerythroaphin*-fb (30 mg.) as deep red plates; these were recrystallised and dried rigorously before analysis (Found: C, 51·3; H, 3·2. $C_{30}H_{20}Br_2O_{10}$ requires C, 51·4; H, 2·9%); $\lambda_{max.}$ (*a*) in 80% aqueous dioxan, 261, 346, 478, 526, and 568 mμ (log ε 4·73, 3·80, 4·60, 4·17, and 4·24), (*b*) in aqueous dioxan containing 10% aqueous sodium hydroxide (1 drop), 470, 586, and 634 mμ; $\nu_{max.}$ in chloroform solution at 3400, 1633, 1603, and 1582 cm.$^{-1}$.

(*b*) Dihydroxyerythroaphin-*fb* (22 mg.) was shaken with a solution of bromine (0·05 ml.) in glacial acetic acid (2 ml.) for 1 hr. The mixture was poured into water (20 ml.), and the precipitate (20 mg.) collected by centrifugation. Recrystallisation from acetone–cyclohexane gave dibromodihydroxyerythroaphin-*fb*, whose ultraviolet and infrared spectra were identical with those of material from (*a*).

Reduction of dibromodihydroxyerythroaphin-*fb* (15 mg.) in the usual way [3] gave erythroaphin-*fb* (6 mg.), whose identity was confirmed by spectral comparison.

Diacetamidodibromoerythroaphin-fb.—Diacetamidoerythroaphin-*fb* [15] (34 mg.) was brominated as in (*b*) above, the precipitated product was extracted into chloroform (10 ml.), washed with aqueous sodium thiosulphate, then water, and dried, and the solvent was evaporated, to yield *diacetamidodibromoerythroaphin*-fb (40 mg.). This recrystallised from chloroform–ethanol (Found: C, 50·9; H, 3·3. Calc. for $C_{34}H_{26}Br_2N_2O_{10},H_2O$: C, 51·0; H, 3·5%) and was identical with the product obtained by acetylating diaminodibromoerythroaphin-*fb* as described in Part XIX.[15]

Conversion of Mono- into Di-hydroxyerythroaphin-fb.—Hydroxyerythroaphin-*fb* (52·7 mg.) and piperidine gave monohydroxymonopiperidinoerythroaphin-*fb* [3] with a total uptake of 1 mol. of oxygen, complete in 11 hr.

The hydroxypiperidino-derivative (47 mg.) in pyridine (7 ml.) was acetylated [15] by adding acetyl chloride (0·8 ml.) at 0°, and setting the whole aside for 15 min. at room temperature. The mixture was then poured into cold 3N-hydrochloric acid (50 ml.) and extracted with chloroform. The extract was washed with dilute hydrochloric acid and then treated briefly with an excess of methanolic sodium hydroxide, thereby hydrolysing ester groups and giving a deep green solution. Extraction with water (50 ml.), acidification of the extract, and re-extraction into chloroform gave a red solution. This was washed and dried, and the solvent evaporated. Recrystallisation of the residue from acetone yielded dihydroxyerythroaphin-*fb* (13 mg.), whose infrared and visible spectra were identical with those of authentic material.

Dibromomonohydroxyerythroaphin-fb.—(*a*) Hydroxyerythroaphin-*fb* (123 mg.) was shaken with a solution of bromine (0·27 ml.) in acetic acid (8 ml.) for 90 min., then poured into water (70 ml.) and centrifuged. The precipitate, a red solid (103 mg.), was washed, dried, and

recrystallised from chloroform–ethanol, yielding *dibromohydroxyerythroaphin*-fb (Found, in material dried at 55°/0·1 mm.: C, 49·6; H, 3·3. $C_{30}H_{20}Br_2O_9,2H_2O$ requires C, 50·0; H, 3·4%), $\lambda_{max.}$ 263, 331, 345, 452, 478, 526, and 568 mμ (log ε 4·65, 3·67, 3·68, 4·41, 4·46, 41·2, and 4·22), $\nu_{max.}$ 3410, 2940, 1628, 1576, 1460, 1372, 1305, 1235, 1158, 1105, 1079, 1051, 976, 945, 856, 847, 826, and 807 cm.$^{-1}$. Paper chromatography in 2 : 1 chloroform–light petroleum saturated with water gave R_F 0·25.

(*b*) Dibromoerythroaphin-*fb* (152 mg.), acetic anhydride (22 ml.), and 60% perchloric acid (2 drops) were stirred at 0° for 24 hr. The pigment gradually went into solution and after 5 hr. a finely divided orange-red solid (91 mg.) had separated (see below). This gradually redissolved. After 24 hr., the mixture was filtered and stirred with saturated aqueous sodium acetate (50 ml.) for 1 hr. Extraction with ether (40 ml.) gave a yellow solution (of leuco-acetate), having a strong green fluorescence ($\lambda_{max.}$ 430, 455, and 490 mμ). After being washed with water and aqueous sodium hydrogen carbonate, it was shaken with 5% methanolic sodium hydroxide (40 ml.). Extraction with water gave a green solution which was acidified and then extracted with chloroform (30 ml.). This extract yielded a dark red powder (28 mg.) after " crystallisation " from acetone–cyclohexane. This could not be further purified and was shown by paper chromatography to be a mixture (R_F 0·02, 0·25, 0·70). A similar mixture containing apparently the same products was also obtained from dibromoerythroaphin-*sl*.

The orange-red intermediate (above) crystallised readily from chloroform–ethanol as red needles. It had $\lambda_{max.}$ 262, 342, 449, 534, and 564 mμ and $\nu_{max.}$ 1787 cm.$^{-1}$. Treatment with a solution of sodium hydroxide in aqueous dioxan yielded dibromoerythroaphin-*fb*, whose identity was confirmed spectroscopically.

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UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, May 2nd,* 1963.]