12. *Colouring Matters of the Aphididæ. Part XIX.*[1] *Further Reactions of the Erythroaphins.*

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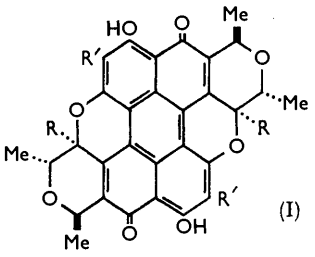
In addition to the erythroaphins-*fb* and -*sl* a third isomer, -*tt* (*trans-trans*), is known. This isomer and several of its derivatives have now been studied in more detail. Its reactions are similar to those of the *sl* isomer, in that it undergoes dihalogenation without, and diamination with, epimerisation to *fb* derivatives. The erythroaphins have been reductively methylated to tetramethyl ethers. Unlike other halogeno-atoms, chloro-substituents in erythroaphins are stable to reduction and tetra-acetyldichlorodihydro- and tetramethyldichlorodihydro-derivatives have been synthesised. Mild acetylation of diaminoerythroaphins yields diacetamido-derivatives, and more vigorous treatment is necessary to acetylate the *peri*-hydroxyl groups.

In Part XVIII of this series[1] we advanced stuctures (I) and (II) (R = R′ = H) for erythroaphins-*fb* and -*sl*, respectively, and showed them to be consistent with the complex reactions these compounds undergo. In the course of this work, which has extended over a number of years, we have examined several other aspects of erythroaphin chemistry, some of which have a direct bearing on structural problems, while others have been undertaken to provide materials for spectroscopic studies. It is these aspects with which the present paper is concerned.

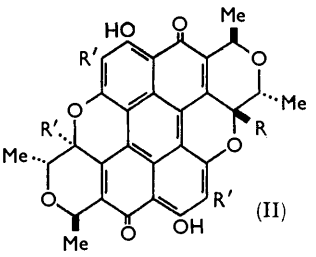
It is evident from a consideration of structures (I) and (II) that, although it has not as yet been found in Nature, a third aphin series could exist the end product of which would be the erythroaphin (III). In Part XIV[2] the interconversion of the isomeric tetra-acetyldihydroerythroaphins-*fb* and -*sl* was described and the isolation of a third

[1] Part XVIII, Cameron, Cromartie, Hamied, Scott, and Todd, preceding paper.

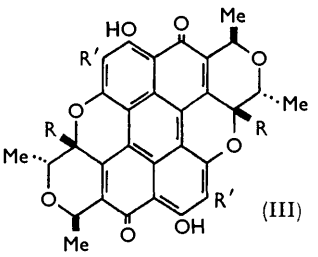
isomer from the irradiation product of tetra-acetyldihydroerythroaphin-*fb* was reported. This new isomer was converted by hydrolysis and oxidation into a new erythroaphin, which had not been obtained from natural sources. This we designated as *trans-trans*-erythroaphin for reasons which are now irrelevant since they related to structures which



(I)



(II)



(III)

have been superseded by (I) and (II), but we shall none the less retain the name erythroaphin-*tt* for convenience in the discussion which follows. In view of the fact that derivatives of erythoaphins-*fb*, -*sl*, and -*tt* appear to co-exist in the irradiated solutions of the tetra-acetyl-*fb* derivative and that no other isomer appears to be present, we now formulate erythroaphin-*tt* as (III; R = R′ = H). The gradation in physical properties along the series *fb* ⟶ *sl* ⟶ *tt* is in accord with this structure, whose chemical properties should be expected to follow a pattern similar to that established for erythroaphin-*sl* (II).

For the preparation of erythroaphin-*tt* in quantity, tetra-acetyldihydroerythroaphin-*sl* proved the most satisfactory starting material. Ultraviolet irradiation of a benzene solution of this acetate followed the course already described,[2] and after 24 hours the mixture contained roughly equal amounts of the *fb* and the *tt* isomer with a smaller amount of the *sl*-compound. The *tt* isomer was obtained pure by repeated fractional crystallisation until the infrared spectrum of the erythroaphin prepared from successive fractions was unchanged and was devoid of the bands at 856 and 976 cm.$^{-1}$, characteristic of erythroaphin-*sl*, the *sl*-isomer being the one most closely associated with it in the crystallisation procedure (the sample of erythroaphin-*tt* described in Part XIV [2] had weak absorption peaks at these frequencies and was contaminated with erythroaphin-*sl*). Pure tetra-acetyldihydroerythroaphin-*tt* has $[\alpha]_D^{20}$ +770°, which is in arithmetic progression with the specific rotations of the *sl* and the *fb* isomer (+95° and −580°, respectively). Erythroaphin-*tt* (III; R = R′ = H) has the same ultraviolet and visible absorption as its two naturally occurring isomers. With piperidine it yields dipiperidinoerythroaphin-*fb* (I; R = piperidino, R′ = H). It yields dibromo- and dichloro-derivatives (III; R = H, R′ = Hal), distinct from the corresponding derivatives of erythroaphin-*fb* and -*sl*, but, like them, converted by the action of piperidine into the corresponding dihalogenodipiperidinoerythroaphin-*fb* (I; R = piperidino, R′ = Hal). The great ease with which erythroaphin-*tt* undergoes hydroxylation prevented us from finding conditions in which it could directly be isomerised to erythroaphin-*fb* without substitution, although this conversion can be achieved with erythroaphin-*sl*. Nevertheless, the pattern of its substitution reactions shows that erythroaphin-*tt* can undergo the base-catalysed isomerisation characteristic of erythroaphin-*sl* on both sides of the molecule, which is consistent with formula (III).

Although diacetyl [3] and dibenzoyl [2] derivatives of the erythroaphins can readily be obtained, attempts to isolate the corresponding dimethyl ethers have been unsuccessful. Methylation can be brought about, but the products appear to be highly photosensitive in solution, changing colour after only a few minutes and no pure material has been obtained. However, the dihydroerythroaphins are readily methylated by dimethyl sulphate and alkali in the absence of air and preferably in the dark or in a diffuse red light, giving orange tetramethyl ethers. The dihydrotetramethylerythroaphins-*fb* and -*sl* are

[2] Brown, Calderbank, Johnson, Quayle, and Todd, *J.*, 1955, 1144.
[3] Human, Johnson, MacDonald, and Todd, *J.*, 1950, 477.

similar in appearance and closely resemble the corresponding tetra-acetyl derivatives [3] in light absoption and in their green fluorescence in solution; they differ markedly, however, in optical rotation and in their infrared spectra. Like the acetates, they mutarotate in organic solvents on exposure to ultraviolet radiation, being converted into a mixture of *fb*, *sl*, and (presumably) *tt* isomers, the last being present in large amount. Although the third tetramethyl ether has not been related by chemical transformations to erythroaphin-*tt* there can be little doubt as to its identity since its specific roation (+517°) is in arithmetical progression with those of the *fb* (−428°) and *sl* (+40°) isomers. Its infrared spectrum differs from those of the other two. All three dihydrotetramethyl-erythroaphins are unaffected by boiling 5% aqueous sodium hydroxide in the dark and in this respect differ markedly from erythroaphin-*sl* and -*tt* which are rapidly converted into compounds of the *fb* series under these conditions. The suppression of the base-catalysed isomerisation of the erythroaphins by reductive methylation shows that the central quinonoid portion of the molecule is essential to the isomerisation, as would be expected on the basis of structures (I—III).

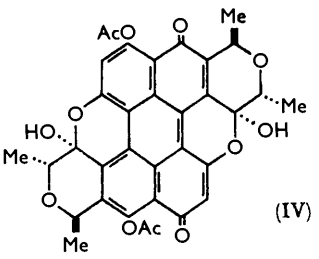
In earlier papers of this series we described numerous examples of halogenoerythro-aphins, containing chlorine, bromine, and iodine. A noteworthy difference between chloro-derivatives and the others is that, in the latter, substituents can readily be removed from the erythroaphin nucleus by reduction, under conditions in which the former are stable. For example, dichloroerythroaphin-*fb* and -*sl* (I, II; R = H, R′ = Cl) can be converted into the same dichlorodipiperidinoerythroaphin-*fb* (I; R = piperidino, R′ = Cl) which on reduction yields dichloroerythroaphin-*fb*. As a consequence of this, reductive methylation of dichloroerythroaphin-*fb* and -*sl* leads to dichlorodihydrotetramethylerythroaphin-*fb* and -*sl*. The properties and visible spectra of these compounds are similar to those of the non-halogenated tetramethyl ethers described above. Their respective specific rotations are comparable (−430° and +55°, respectively, for the dichlorotetramethyl compounds) and they mutarotate on irradiation in ultraviolet light. They are stable to boiling 15% aqueous sodium hydroxide. Tetra-acetyldichlorodihydroerythroaphin-*fb* and -*sl* have also been prepared by standard methods. Their properties are as expected and on cautious alkaline hydrolysis they yield their respective dichloroerythroaphins. These dichloro-tetramethyl and tetra-acetyldichloro-derivatives were of particular structural value. Unlike the dichloroerythroaphins they are soluble enough for nuclear magnetic resonance (n.m.r.) spectroscopy and were used to show the absence of aromatic CH protons in dihalogenated erythroaphins.[4] Using standard procedures [5,6] we have also prepared diaminodichloro- and dichlorodihydroxy-erythroaphin-*fb* (I; R = NH_2 or OH, R′ = Cl); their properties are as would be expected.

We consider finally some experiments involving the acetylation of erythroaphin deriv-atives under different conditions (in doing so we exclude the results of Thiele acetoxyl-ation, which have been discussed in Part XVIII [1]). Acetylation of the *peri*-hydroxyl groups in simple erythroaphins so as to yield diacetyl derivatives is readily effected by acetyl chloride in pyridine [3] and we record in the Experimental section the preparation of diacetyldichloro- and diacetyldibromo-erythroaphin-*sl* in this way. However, acetylation of amino- and hydroxy-erythroaphins (*e.g.*, I; R = NH_2 or OH) can be brought about in two separable stages. If simply warmed with acetic anhydride alone these derivatives are converted into the corresponding diacetamido- and diacetoxy-compounds (I; R = NHAc or OAc). Of these, the latter in general are difficult to purify and of little value, but the former crystallise well and are useful for characterising the amino-compounds, some of which retain organic solvents even *in vacuo* up to the temperature at which they begin to decompose. The diacetamido-compounds, being relatively soluble, have also proved useful for n.m.r. spectroscopic studies and for structural work, *e.g.*, in Part XVIII [1]

[4] Part XXI, Cameron, Cromartie, Hamied, Scott, Sheppard, and Todd, *J.*, 1964, 90.
[5] Brown, Johnson, MacDonald, Quayle, and Todd, *J.*, 1952, 4928.
[6] Brown, Calderbank, Johnson, MacDonald, Quayle, and Todd, *J.*, 1955, 954.

where bromination of diacetamidoerythroaphin-*fb* (I; R = NHAc, R′ = H) yielded the diacetamidodibromo-derivative (I; R = NHAc, R′ = Br). This dibromo-compound had previously been obtained by acetylating diaminodibromoerythroapin-*fb* (I; R = NH_2, R′ = Br). Such diacetamido- and diacetoxy-compounds can also be prepared, if less conveniently, by acetylation of the amino- and hydroxy-starting materials with acetyl chloride in pyridine, the *peri*-hydroxyl groups being also acetylated, followed by selective alkaline hydrolysis.

When piperidino-substituted erythroaphins are treated with acetyl chloride in pyridine at room temperature a completely different reaction ensues. Pouring there action mixture into dilute acid precipitates almost immediately a derivative of the corresponding hydroxy-compound. For example, dipiperidinoerythroaphin-*fb* (I; R = piperidino, R′ = H) has been reported to give in 60% yield diacetyldihydroxyerythroaphin-*fb* [2] (IV), which is readily hydrolysed to dihydroxyerythroaphin-*fb* (I; R = OH, R′ = H). A similar example has also been described in Part XVIII [1] in considering the chemistry of monohydroxyerythroaphin-*fb*. The ease with which this reaction occurs is striking and, while we have not studied it in detail, it seems consistent with the structures we have proposed for erythroaphin derivatives. For example, quaternisation of the piperidino-groups would facilitate fission of the bond joining the piperidino-group to the erythroaphin system with the formation of *N*-acetylpiperidine and a mesomeric carbonium ion which, in aqueous solution, would give rise to the hydroxy-compound.



(IV)

Experimental

Unless otherwise stated, ultraviolet and visible spectra were measured for chloroform solutions, and infrared spectra for Nujol and/or hexachlorobutadiene mulls.

Irradiation of Tetra-acetyldihydroerythroaphin-sl.—All operations except the actual irradiation were carried out in a subdued red light and crystallisations were allowed to proceed in the dark. Optical rotations were measured throughout for benzene solutions. Unless otherwise stated, light petroleum refers to the fraction of b. p. 40—60°. A solution of tetra-acetyldihydroerythroaphin-*sl* (950 mg.; $[\alpha]^{20}$ +35°) in benzene (800 ml.) was irradiated in a silica flask with ultraviolet light from a mercury lamp for 24 hr. at room temperature, then concentrated to 100 ml. and poured on a column of silica gel (5 × 15 cm.). Elution with 19 : 1 bezene–ethyl acetate (4 l.) gave a yellow solution which was concentrated to 200 ml., diluted with an equal volume of light petroleum, and set aside for 3 days. Tetra-acetyldihydroerythroaphin-*fb* separated and was filtered off. This process was repeated several times until a total of 4 g. of tetra-acetyldihydroerythroaphin-*sl* had been irradiated. The total yield of the crystalline *fb*-isomer was 1·4 g.

Tetra-acetyldihydroerythroaphin-tt. The combined mother-liquors (1·5 l.) left after separation of the *fb*-isomer in the above experiments were concentrated to 100 ml., diluted with an equal volume of light petroleum, and set aside for 2 days and the crystalline material (100 mg.; fraction A) was collected. The filtrate from A was then concentrated to 30 ml. and diluted with light petroleum (120 ml.), the immediately precipitated orange-brown solid (900 mg.; fraction B) was collected, and the filtrate left to deposit overnight a further quantity of crystals (300 mg.; fraction C). After collection of fraction C, concentration to 15 ml. and dilution with light petroleum (150 ml.) gave after 2 days a new crop of brownish crystals (100 mg.; fraction D), and repetition by concentration of the filtrate from D to 10 ml. and dilution with light petroleum (120 ml.) gave fraction E (140 mg.) as orange nodules. A final fraction F (80 mg.) was obtained by concentrating the filtrate from E to 5 ml., adding light petroleum (75 ml.), and setting the mixture aside for 3 weeks.

The composition of each fraction was assessed by examining the infrared spectra of the erythroaphins prepared from a sample of each of them in the following way: A solution of the material in chloroform was shaken with a 1% solution of sodium hydroxide in 85% aqueous

methanol for 1 min. The resulting green mixture was at once acidified with 10% hydrochloric acid and shaken so that the erythroaphin liberated passed into the chloroform layer; this was thoroughly washed, then dried, concentrated to small bulk, and diluted with hot ethanol, which caused crystallisation of the erythroaphin. In a control experiment it was shown that under these conditions tetra-acetyldihydroerythroaphin-*sl* was converted into erythroaphin-*sl* without concomitant production of the *fb* isomer.

Fraction A consisted wholly of the *fb* isomer. B was a mixture of the *sl* and *tt* compounds. C, D, E, and F gave erythroaphins of identical infrared spectra in which the 856 and 976 cm.$^{-1}$ bands of the *sl* isomer were absent; they were considered to be essentially pure *tt* material.

Fractions C and D were combined and dissolved in hot benzene (33 ml.), and light petroleum (125 ml.) was added. The solution slowly deposited brownish needles (250 mg.; fraction G), and the filtrate from these, concentrated to 6 ml. and diluted with light petroleum (100 ml.), deposited during 2 days orange-yellow crystals (110 mg.; fraction H). By combining fractions B and G and fractionation as before a further group of fractions was obtained, *viz.*: I, 400 mg., containing *sl* and *tt*; J, 300 mg.; and K, 300 mg. Fractions J and K contained only the *tt* isomer.

Fraction H was twice recrystallised from ethanol containing a few drops of chloroform and gave orange crystals of *tetra-acetyldihydroerythroaphin*-tt, $[\alpha]_D^{20}$ + 770° (*c* 0·03 in benzene). When heated it charred but did not melt (Found: C, 67·2; H, 5·1. $C_{38}H_{30}O_{12}$ requires C, 66·9; H, 5·0%); it had $\lambda_{max.}$ 281, 357, 413, 438, 465, and 502 mμ (log ε, 4·55, 3·55, 3·70, 4·03, 4·31, and 4·43), and $\nu_{max.}$ 1765, 1621, 1604, 1587, 1505, 1333, 1268, 1189, 1150, 1108, 1073, 1031, 1010, 970, 884, 843, 820, and 675 cm.$^{-1}$.

Erythroaphin-tt.—Hydrolysis of fractions E, F, and K (520 mg. in all) with aqueous-methanolic sodium hydroxide as described under I (above) yielded erythroaphin-*tt* (340 mg.), that formed dark red crystals from chloroform–ethanol (Found: C, 70·9; H, 4·4. Calc. for $C_{30}H_{22}O_8$: C, 70·3; H, 4·7%). It had $\lambda_{max.}$ 253, 422, 448, 488, 524, 565, and 590 mμ (log ε, 4·49, 4·39, 4·50, 3·70, 4·03, 4·22, and 3·84), and $\nu_{max.}$ 1624, 1587, 1350, 1281, 1257, 1215, 1186, 1165, 1113, 1078, 1045, 998, 964, 867, 827, 812, 766, 737, and 687 cm.$^{-1}$. On paper chromatography in 3 : 20 chloroform–light petroleum (b. p. 100—120°) it had R_F 0·77; in the same system erythroaphin-*fb* had R_F 0·37 and erythroaphin-*sl* R_F 0·75.

Dipiperidinoerythroaphin-fb *from Erythroaphin*-tt.—A solution of erythroaphin-*tt* (20 mg.) in pyridine (6 ml.) containing piperidine (0·6 ml.) was set aside overnight, poured into ice-cold 10% hydrochloric acid, and extracted with chloroform. The extract was washed with dilute hydrochloric acid and then shaken with concentrated hydrochloric acid (3 × 30 ml.) which extracted the pigment. The acid layer was diluted with water (110 ml.) and again extracted with chloroform, and the extract was washed with water and sodium hydrogen carbonate, dried, and evaporated. The residue recrystallised from pyridine–methanol as red plates (11 mg.) of dipiperidinoerythroaphin-*fb*, spectrally identical with an authentic specimen.

Dibromoerythroaphin-tt.—Erythroaphin-*tt* (30 mg.) was brominated in the usual way and the product recrystallised from chloroform–ethanol to yield tiny, almost black crystals of dibromoerythroaphin-*tt*, $\lambda_{max.}$ 263, 452, 527, and 569 mμ (log ε 4·71, 4·49, 4·21, and 4·34), $\lambda_{infl.}$ 478 mμ (log ε 4·41), and $\nu_{max.}$ 1620, 1580, 1350, 1302, 1272, 1240, 1203, 1167, 1138, 1112, 1082, 1042, 1001, 967, 887, 860, 843, 825, 808, 795, and 763 cm.$^{-1}$. Although insufficient pure material was available for analysis, the structure was confirmed by treatment with piperidine as above, which yielded dibromodipiperidinoerythroaphin-*fb*, spectroscopically identical with an authentic sample.

Dichloroerythroaphin-tt.—Prepared in the usual way from erythroaphin-*tt* (40 mg.), *dichloro-erythroaphin*-tt (15 mg.) formed dark red crystals from chloroform–ethanol (Found: C, 61·7; H, 3·9. $C_{30}H_{20}Cl_2O_8$ requires C, 62·2; H, 3·5%); it had $\lambda_{max.}$ 262, 345, 450, 525, and 567 mμ (log ε 4·42, 3·59, 4·43, 4·13, and 4·26), and $\nu_{max.}$ 1622, 1580, 1350, 1300, 1275, 1245, 1204, 1168, 1140, 1113, 1085, 1045, 1001, 975, 927, 907, 852, 827, 812, 800, and 763 cm.$^{-1}$.

Dichlorodipiperidinoerythroaphin-fb.—(I) Dichloroerythroaphin-*fb* (180 mg.) was dissolved in pyridine (30 ml.) containing piperidine (3 ml.), and the solution was left overnight and then worked up as described for the dibromo-compound. Recrystallised from chloroform–ethanol *dichlorodipiperidinoerythroaphin*-fb formed dark red crystals (106 mg.) (Found, in material dried at 75°/10^{-3} mm.: C, 64·7; H, 5·2. $C_{40}H_{38}Cl_2N_2O_8$ requires C, 64·4; H, 5·1%), $\lambda_{max.}$ 264, 494, 533, and 578 mμ (log ε, 4·56, 4·32, 4·28, and 4·33), $\nu_{max.}$ 1623, 1567, 1282, 1250, 1215, 1190, 1175, 1160, 1135, 1107, 1074, 1037, 1020, 973, 953, 905, 867, 850, 807, 790, and 767 cm.$^{-1}$.

(II) Dichloroerythroaphin-*sl*, treated in the same way, gave a product (Found: C, 64·2; H, 5·1%), identical in ultraviolet and infrared spectra with the product obtained as in (I).

(III) The same product (identical spectra) was obtained by similar treatment of dichloroerythroaphin-*tt*.

Dichloroerythroaphin-fb *from Dichlorodipiperidinoerythroaphin*-fb.—The dichlorodipiperidino-compound (15 mg.) was reduced for 40 min. with zinc dust and acetic acid in the usual way, yielding dichloroerythroaphin-*fb*, whose spectra were identical with those of authentic material.

Dihydrotetramethylerythroaphin-sl.—All operations in this preparation were carried out wherever possible in the dark or, failing that, in a diffuse red light.

Erythroaphin-*sl* (750 mg.) was hydrogenated at room temperature and atmospheric pressure in dioxan (150 ml.) with Adams platinum catalyst (300 mg.). 10% Aqueous sodium hydroxide (30 ml.) was added (changing the green-fluorescent orange colour of the solution to magenta) and then dimethyl sulphate (18 ml.), and shaking was continued overnight under hydrogen. The resulting orange solution was poured on ice, and the precipitate was collected, dissolved in benzene (75 ml.), and put on a column of silica gel (5 × 12 cm.). The main orange band on the column was eluted with benzene–ethyl acetate (19 : 1), the eluate evaporated and dissolved in a minimum of hot chloroform, and ethanol was added. *Dihydrotetramethylerythroaphin*-sl (400 mg.) separated as orange plates. Recrystallised from chloroform–ethanol it had m. p. 232—233° (decomp.), $[\alpha]_D^{20}$ +40° (*c* 0·050 in benzene) (Found: C, 72·0; H, 5·6; OMe, 21·5. $C_{34}H_{32}O_8$ requires C, 71·8; H, 5·7; OMe, 21·8%), $\lambda_{max.}$ 265, 277, 347, 363, 390, 413, 439, 468, and 503 mμ (log ε 4·50, 4·44, 3·52, 3·74, 3·32, 3·55, 4·02, 4·39, and 4·54), and $\nu_{max.}$ 1622, 1585, 1505, 1435, 1420, 1367, 1356, 1323, 1300, 1295, 1265, 1213, 1193, 1182, 1137, 1116, 1101, 1081, 1043, 1016, 990, 965, 956, 947, 927, 877, 841, 820, 800, 740, and 685 cm.$^{-1}$. The substance was soluble in chloroform, benzene, and dioxan, giving orange solutions with a strong green fluorescence, but sparingly soluble in ethanol and light petroleum. It was unchanged (infrared spectrum) when heated at 100° for 35 min. with a 5% solution of sodium hydroxide in aqueous dioxan and at room temperature for 7 days in the dark. It was very sensitive to acids; its solution in dioxan slowly became deep green after addition of a few drops of concentrated hydrochloric acid, and it dissolved in 70% sulphuric acid to give a violet solution from which it could not be recovered by dilution.

Dihydrotetramethylerythroaphin-fb.—Reductive methylation of erythroaphin-*fb* according to the procedure above described for the *sl* isomer gave *dihydrotetramethylerythroaphin*-fb, orange-yellow needles (from chloroform–ethanol), m. p. 255° (decomp.), $[\alpha]_D^{20}$ −430° (*c* 0·042 in benzene) (Found: C, 71·4; H, 5·8; OMe, 21·9%), $\lambda_{max.}$ 279, 347, 363, 390, 415, 440, 470, and 505 mμ (log ε 4·54, 3·50, 3·72, 3·34, 3·56, 4·03, 4·38, and 4·57), $\nu_{max.}$ 1622, 1586, 1505, 1422, 1357, 1322, 1287, 1262, 1212, 1182, 1145, 1135, 1107, 1078, 1050, 1015, 992, 980, 965, 945, 930, 865, 825, 817, 802, 754, and 675 cm.$^{-1}$. It was similar to the *sl* isomer in its general properties but its solutions in organic solvents appeared to be more intensely fluorescent.

Dihydrotetramethylerythroaphin-tt.—Dihydrotetramethylerythroaphin-*sl* (65 mg.) was dissolved in benzene (120 ml.) in a silica flask and irradiated with ultraviolet light from a mercury lamp for 24 hr. Solvent was removed under reduced pressure and the residue crystallised from chloroform–ethanol (59 mg.). Two further recrystallisations gave reddish-orange crystals of *dihydrotetramethylerythroaphin*-tt, m. p. 180—182° (decomp.), $[\alpha]_D^{20}$ +517° (*c* 0·018 in benzene) (Found: C, 72·0; H, 5·9; OMe, 20·7%), $\lambda_{max.}$ 265, 278, 347, 363, 390, 415, 440, 470, and 504 mμ (log ε 4·44, 4·50, 3·57, 3·74, 3·40, 3·57, 4·01, 4·38, and 4·52), $\nu_{max.}$ 1621, 1584, 1506, 1420, 1360, 1327, 1307, 1282, 1265, 1210, 1180, 1150, 1135, 1115, 1090, 1080, 1048, 1013, 965, 886, 820, and 695 cm.$^{-1}$. The compound was similar in its properties to the *sl* and *fb* isomers but its solutions were less strongly fluorescent; like them it was unchanged (infrared spectrum) by a 5% solution of sodium hydroxide in aqueous dioxan at 100° for 35 min.

Dichlorodihydrotetramethylerythroaphin-sl.—Dichloroerythroaphin-*sl* (250 mg.) was reductively methylated as above and yielded *dichlorodihydrotetramethylerythroaphin*-sl (106 mg.) as orange crystals (two crystallisations from chloroform–ethanol), $[\alpha]_D^{20}$ +55° (*c* 0·036 in benzene) (Found: C, 63·7; H, 5·1; Cl, 11·5; OMe, 18·6. $C_{34}H_{30}Cl_2O_8$ requires C, 64·0; H, 4·7; Cl, 11·3; OMe, 19·3%), $\nu_{max.}$ 287, 367, 442, 470, and 504 mμ (log ε 4·60, 3·14, 3·93, 4·37, and 4·52), $\nu_{max.}$ 1567, 1500, 1423, 1367, 1324, 1264, 1195, 1148, 1136, 1105, 1080, 1048, 1011, 970, 958, 949, 930, 903, 893, 871, 843, 800, and 755 cm.$^{-1}$. It was recovered unchanged (infrared spectrum) after 3 hours' boiling with a 15% solution of sodium hydroxide in aqueous dioxan.

Dichlorodihydrotetramethylerythroaphin-fb.—Similar treatment of dichloroerythroaphin-*fb*

(170 mg.) yielded, after two recrystallisations from chloroform–ethanol, *dichlorodihydrotetramethylerythroaphin-fb* (70 mg.) as orange crystals, $[\alpha]_D^{20}$ −430° (*c* 0·021 in benzene) (Found: C, 64·2; H, 5·1%), $\lambda_{max.}$ 287, 367, 416, 440, 470, and 503 mμ (log ε, 4·61, 3·19, 3·49, 3·93, 4·37, and 4·52), $\nu_{max.}$ 1615, 1568, 1495, 1322, 1262, 1196, 1135, 1118, 1101, 1080, 1050, 1014, 975, 949, 901, 881, 840, 827, 815, 795, 764, 754, 744, and 698 cm.$^{-1}$. A dilute solution irradiated in benzene for 24 hr. gave an orange solution, having a faint green fluorescence and an optical rotation corresponding to $[\alpha]_D^{20}$ *ca.* +570°.

Tetra-acetyldichlorodihydroerythroaphin-sl.—The whole procedure was carried out in subdued light and crystallisation allowed to proceed in the dark. A solution of dichloroerythroaphin-*sl* (100 mg.) and fused sodium acetate (30 mg.) in acetic anhydride (10 ml.) was hydrogenated for 1·5 hr. at room temperature in the presence of Adams catalyst (27 mg.). The solution was filtered and the acetic anhydride evaporated under reduced pressure. The residue was boiled with ethanol (30 ml.) for 5 min., then evaporated, and the remaining solid was triturated with water, filtered off, and washed with ethanol. It was then dissolved in benzene and applied to a column of silica (2 × 8 cm.). Elution with benzene–ethyl acetate (19 : 1) gave a yellow solution having a green fluorescence. Evaporation of this solution gave an orange gum, which, twice crystallised from chloroform–ethanol, yielded *tetra-acetyldichlorodihydroerythroaphin*-sl (25 mg.) as yellow needles (Found: C, 61·2; H, 4·1. $C_{38}H_{30}Cl_2O_{12}$ requires C, 60·9; H, 4·0%), $\lambda_{max.}$ 283, 363, 411, 437, 466, and 498 mμ (log ε, 4·63, 3·18, 3·52, 4·01, 4·38, and 4·51), $\nu_{max.}$ 1775, 1617, 1585, 1570, 1320, 1265, 1190, 1132, 1120, 1095, 1040, 1005, 963, 915, 875, 840, 825, 785, 767, and 720 cm.$^{-1}$. A sample (9 mg.), when hydrolysed with methanolic sodium hydroxide as for tetra-acetyldihydroerythroaphin-*sl*, yielded dichloroerythroaphin-*sl* (3 mg.), whose identity was confirmed by spectral comparison.

Tetra-acetyldichlorodihydroerythroaphin-fb.—Dichloroerythroaphin-*fb* (65 mg.) was reductively acetylated as described above for the *sl* isomer. Two crystallisations from chloroform–ethanol gave *tetra-acetyldichlorodihydroerythroaphin*-fb (35 mg.) as yellow needles (Found: C, 61·3; H, 4·2. $C_{38}H_{30}Cl_2O_{12}$ requires C, 60·9; H, 4·0%), $\lambda_{max.}$ 285, 363, 436, 467, and 498 mμ (log ε 4·64, 3·27, 4·03, 4·39, and 4·53), $\nu_{max.}$ 1778, 1590, 1570, 1323, 1275, 1264, 1185, 1135, 1122, 1075, 1041, 1014, 963, 915, 870, 840, 825, 745, and 700 cm.$^{-1}$. A sample (10 mg.), hydrolysed as above for the *sl* isomer, yielded dichloroerythroaphin-*fb* (4 mg.), whose identity was confirmed by spectral comparison.

Diaminodichloroerythroaphin-fb.—(I) Dichloroerythroaphin-*fb* (510 mg.) was aminated as for the dibromo-compound,[6] and the resulting solid (439 mg.) recrystallised from pyridine–methanol–10% hydrochloric acid (10 : 7 : 3). *Diaminodichloroerythroaphin*-fb crystallised well, forming dark red needles with a green lustre, but it appeared to retain pyridine of crystallisation which could not be removed and prevented a satisfactory analysis. It was therefore characterised by conversion into its diacetamido-derivative (see below). Diaminodichloroerythroaphin-*fb* had ultraviolet max. at 261, 333, 347, 477, 526, and 568 mμ [log ε (on the basis of $C_{30}H_{22}Cl_2N_2O_8,C_5H_5N$) 4·62, 3·66, 3·67, 4·45, 4·11, and 4·21), and infrared absorption at 3370, 3310, 2930, 1630, 1573, 1453, 1368, 1310, 1284, 1243, 1187, 1160, 1103, 1086, 1068, 1053, 1028, 1000, 940, 862, 812, 787, 743, and 701 cm.$^{-1}$.

(II) Dichloroerythroaphin-*sl* (370 mg.), treated as above, also gave diaminodichloroerythroaphin-*fb* (280 mg.) on the basis of spectral comparisons.

Diacetamidodichloroerythroaphin-fb.—Diaminodichloroerythroaphin-*fb* (138 mg.) was warmed with acetic anhydride (25 ml.) for 1 hr. Solvent was removed under reduced pressure and *diacetamidodichloroerythroaphin*-fb obtained; it formed chunky red needles (86 mg.) from chloroform–ethanol (Found, in material dried at 60°/0·1 mm.: C, 57·3; H, 4·4; N, 3·7. $C_{34}H_{26}Cl_2N_2O_{10},H_2O$ requires C, 57·4; H, 4·0; N, 3·9%). It had $\lambda_{max.}$ 263, 333, 478, 529, and 571 mμ (log ε 4·63, 3·75, 4·49, 4·14, and 4·19), and $\nu_{max.}$ 3370, 3350, 3000, 2960, 1680, 1628, 1600, 1575, 1455, 1370, 1304, 1245, 1165, 1155, 1100, 1085, 1073, 1056, 997, 990, 960, 930, 878, 865, 848, 830, and 749 cm.$^{-1}$.

Dichlorodihydroxyerythroaphin-fb.—Diaminodichloroerythroaphin-*fb* (142 mg.) was treated with nitrous acid in the usual way.[5] The resulting solid (119 mg.) recrystallised from acetone–benzene–cyclohexane, forming red needles of *dichlorodihydroxyerythroaphin*-fb. As with the diamino-compound above, this material retained organic solvents tenaciously and an analytical specimen dried at 80°/10^{-4} mm. showed a strong infrared band at 1703 cm.$^{-1}$, due to acetone while its n.m.r. spectrum (40 Mc./sec.) had bands at τ 2·65 (benzene) and 7·87 (acetone). More vigorous drying, at higher temperatures, appeared (infrared and analysis) to remove acetone,

but non-stoicheiometric amounts of benzene persisted and very variable analyses were obtained on the same sample, depending on drying conditions [Found, on the sample dried at 80°/10^{-1} mm.: C, 62·7; H, 4·0. $C_{30}H_{20}Cl_2O_{10},CO(CH_3)_2,C_6H_6$ requires C, 62·7; H, 4·3%). Light-absorption: max. were at 257, 331, 344, 446, 469, 527, and 570 mμ (log ε 4·66, 3·68, 3·68, 4·45, 4·53, 4·13, and 4·26), and infrared absorption at 3330, 1703 (acetone), 1633, 1570, 1242, 1160, 1120, 1098, 1085, 1075, 1057, 1029, 1017, 978, 957, 948, 867, 832, 682, and 665 cm.$^{-1}$.

Acetylation of Dihalogenoerythroaphins.—(I) Dibromoerythroaphin-*sl* (200 mg.) was acetylated as described above. Recrystallisation from benzene–light petroleum (b. p. 40—60°) gave *diacetyldibromoerythroaphin*-sl (60 mg.) as an orange solid (Found: C, 53·9, H, 3·6. $C_{34}H_{24}Br_2O_{10}$ requires C, 54·3; H, 3·2%), $\lambda_{max.}$ 264, 328, 361, and 450 mμ (log ε 4·37, 4·01, 3·92, and 4·41), $\lambda_{infl.}$ 275 mμ (log ε 4·28), $\nu_{max.}$ 2990, 2940, 1778, 1637, 1600, 1572, 1552, 1450, 1370, 1332, 1264, 1252, 1173, 1113, 1084, 1047, 1006, 957, 866, 849, 833, 800, and 716 cm.$^{-1}$.

(II) Similar treatment of dichloroerythroaphin-*sl* (80 mg.) gave a similar product, presumably diacetyldichloroerythroaphin-*sl* (19 mg.), $\lambda_{max.}$ 262, 330, 360, and 445 mμ, $\nu_{max.}$ 3350, 1776, 1639, 1598, 1575, 1555, 1330, 1310, 1255, 1175, 1110, 1095, 1045, 1010, 960, 935, 886, 870, 830, and 755 cm.$^{-1}$.

Diacetamidoerythroaphin-fb.—(I) Diaminoerythroaphin-*fb* (90 mg.) was acetylated with acetic anhydride by the method described above for the diaminodichloro-compound. Recrystallisation from ethanol yielded *diacetamidoerythroaphin*-fb as reddish-brown needles (88 mg.) (Found, in material dried at 55°/0·1 mm.: C, 63·8, 63·4; H, 5·0, 5·0; N, 4·3. $C_{34}H_{28}N_2O_{10},H_2O$ requires C, 63·6; H, 4·7; N, 4·4%), $\lambda_{max.}$ 255, 322, 433, 460, 529, 570, and 600 mμ (log ε 4·56, 3·61, 4·40, 4·54, 4·05, 4·22, and 3·81), $\nu_{max.}$ 3460, 3320, 2960, 1687, 1635, 1552, 1485, 1370, 1277, 1254, 1235, 1200, 1161, 1085, 1067, 1052, 1008, 937, 865, 850, 835, and 818 cm.$^{-1}$. The compound could be extracted from chloroform into concentrated hydrochloric acid with difficulty, but not into dilute hydrochloric acid; it could be extracted into 10% sodium hydroxide solution, giving a green aqueous layer.

(II) Diaminoerythroaphin-*fb* (105 mg.) in dry pyridine (15 ml.) was acetylated with acetyl chloride (1·6 ml.) at 0°. The mixture was left 15 min. and then poured into ice-cold dilute hydrochloric acid. After 5 min. it was extracted into chloroform (10 × 10 ml.), the organic layer was washed and dried, and the solvent evaporated to give an orange solid. Without purification this was hydrolysed for 15 min. at room temperature with methanolic ammonia (10 ml.). Acidification and extraction into chloroform yielded diacetamidoerythroaphin-*fb* (50 mg.) identical in spectroscopic and paper chromatographic behaviour with the product from (I) above.

Diacetamidodibromoerythroaphin-fb.—Acetylation of diaminodibromoerythroaphin-*fb* (95 mg.) with acetic anhydride as described for the diaminodichloro-compound yielded *diacetamidodibromoerythroaphin*-fb (55 mg.) as red needles (from ethanol) (Found, on material dried at 60°/0·1 mm.: C, 50·7; H, 3·5; N, 3·6. $C_{34}H_{26}Br_2N_2O_{10},H_2O$ requires C, 51·0; H, 3·5; N, 3·5%), $\lambda_{max.}$ 264, 340, 483, 532, and 574 mμ (log ε 4·60, 3·67, 4·49, 4·14, and 4·21), $\nu_{max.}$ 3380, 3260, 3010, 2960, 1683, 1625, 1575, 1540, 1455, 1370, 1240, 1170, 1158, 1101, 1085, 1072, 1060, 1015, 993, 950, 931, 885, 860, 830, 818, 750, and 665 cm.$^{-1}$. It could be extracted from chloroform into 10% sodium hydroxide solution, giving a green colour in the aqueous layer, and was identical with material obtained by bromination of diacetamidoerythroaphin-*fb*.[1]

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