1279. *Colouring Matters of the Aphididae. Part XXVI.*[1] *The Chrysoaphins-sl and their Reaction with Periodate*

By D. W. Cameron, R. I. T. Cromartie, Y. K. Hamied, E. Haslam, D. G. I. Kingston, Lord Todd, and J. C. Watkins

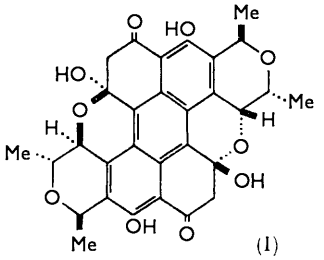
Treatment of chrysoaphin-*sl*-1 with periodate in the presence of benzoyl peroxide yields as the major product a dihydroxychrysoaphin-*sl*-1, formulated tentatively as (III; R, R′ = OH; R″ = H). This compound, though indistinguishable chromophorically from a chrysoaphin, is much more stable towards acid or alkali; it cannot be converted into an erythroaphin under these conditions. However, if first reduced, *e.g.*, with zinc and acetic acid, it readily yields a hydroxyerythroaphin-*sl* (II; R = OH), having a hydroxyl substituent attached directly to the chromophore. Other reactions of these compounds are discussed. Since the isomeric chrysoaphins-*sl*-2 and -*fb* on periodate treatment are not converted into dihydroxychrysoaphins, it is concluded that chrysoaphins-*sl*-1 and -*sl*-2 most probably have structures (III; R, R′, R″ = H) and (IV; R, R′ = H), respectively.

In Part XX [2] it was recorded that dehydration of xanthoaphin-*sl* (I) under very mild conditions led to comparable amounts of two isomeric chrysoaphins. These were distinguishable paper chromatographically and were designated as chrysoaphin-*sl*-1 and -*sl*-2. Each could be converted into one and the same erythroaphin-*sl* (II; R = H). This was explained by the fact that xanthoaphin-*sl* is unsymmetrical; two chrysoaphins (III; R, R′, R″ = H) and (IV; R, R′ = H) may therefore be formed, depending on which of the hemiketal hydroxyl groups is lost during dehydration. Which of these structures represents chrysoaphin-*sl*-1 and which -*sl*-2 was not determined because of the ease of dehydration to erythroaphin-*sl*. Indeed, so readily does this proceed that, apart from the reaction now to be discussed, no transformation of xanthoaphins or chrysoaphins has ever been observed which did not involve it. The *fb* series, on the other hand, is much simpler. In xanthoaphin-*fb* (V) the hemiketal hydroxyl groups are symmetrically placed and only one chrysoaphin-*fb* (VI) and one erythroaphin-*fb* (VII; R = H) are obtained.

From the mixed chrysoaphins-*sl*, produced as above, only the -*sl*-1 isomer has been obtained pure by fractional crystallisation. The *sl*-2 isomer, may, however, be obtained after treating the mixture with sodium metaperiodate in aqueous dioxan.[2] Surprisingly it is relatively stable to these conditions whereas its isomer is largely oxidised. Since this remarkable reaction not only affords a method for obtaining chrysoaphin-*sl*-2 but also distinguishes between it and the *sl*-1 isomer structurally, its course has been examined in greater detail, as has the action of periodate on other aphins. Similar but more prolonged treatment of erythroaphin-*sl* (II; R = H) has been reported [3] to yield hydroxyerythroaphin-*fb* (VII; R = OH), together with presumably more highly oxygenated derivatives which were not isolated. Erythroaphin-*fb* gives essentially the same mixture. In view of this the behaviour of chrysoaphins-*fb* (VI) and -*sl*-2 is unexceptional and is consistent with their being slowly converted into the respective erythroaphins, which then undergo slow oxidation as above. Chrysoaphin-*sl*-1, to some extent, yields similar products, but chiefly it undergoes a more rapid oxidation, not observed in the case of its isomers, to give a new substance, $C_{30}H_{24}O_{11}$, in yields of up to 50%. For ease of subsequent discussion we designate this as compound A. (It is noteworthy that the rate of acid-catalysed dehydration of chrysoaphin-*sl*-1 does not differ appreciably from those of chrysoaphins-*fb* and -*sl*-2. This confirms that the stereospecific production of compound A from the *sl*-1 isomer is indeed due to susceptibility of the latter to oxidation and not, for

[1] Part XXV, J. H. Bowie and D. W. Cameron, *J.*, 1965, 5651.
[2] A. Calderbank, D. W. Cameron, R. I. T. Cromartie, Y. K. Hamied, E. Haslam, D. G. I. Kingston, Lord Todd, and J. C. Watkins, *J.*, 1964, 80.
[3] A. W. Johnson, Sir Alexander R. Todd, and J. C. Watkins, *J.*, 1956, 4091.

example, to slowness of the main side reaction, *viz.*, dehydration to erythroaphin-*sl* and subsequent oxidation.) On oxidation of mixed chrysoaphins-*sl*, conditions can be found in which the *sl*-1 isomer is largely converted into compound A, while chrysoaphin-*sl*-2 is substantially unchanged. A chromatographic separation can then readily be effected, for although the two chrysoaphins cannot be separated from one another in this way, each can be separated from compound A.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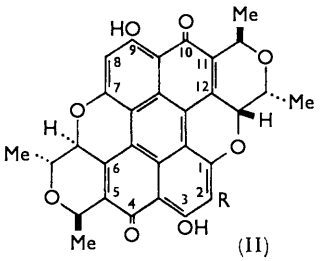
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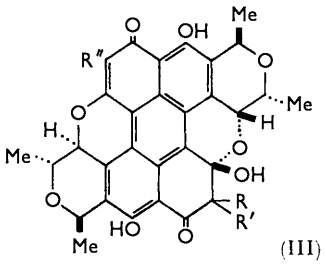
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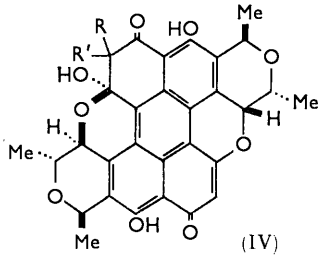
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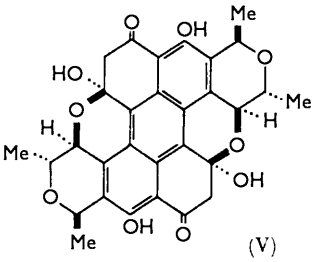
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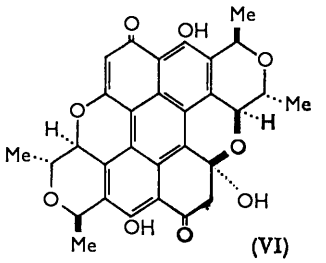
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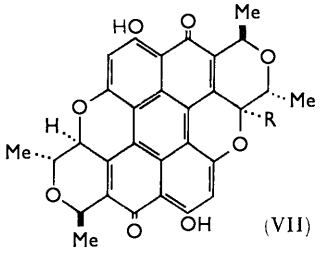
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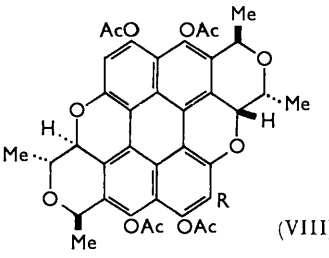
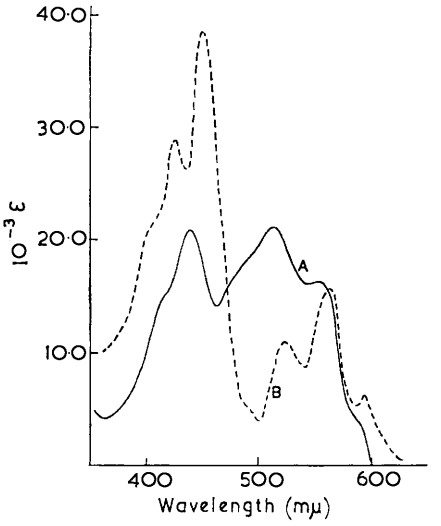

(VIII)

The formula of compound A, $C_{30}H_{24}O_{11}$, differs from that of a chrysoaphin only by two oxygens. Its chromophore is undoubtedly that of a chrysoaphin, as is that of the mono-bromo-derivative into which it is readily converted by the action of bromine in acetic acid. However one of its most striking properties is stability to acid or alkaline conditions such as would rapidly and quantitatively convert a chrysoaphin into the corresponding erythroaphin. Under vigorous or prolonged treatment with acid it undergoes very slow decomposition into unidentified substances, but it can still be detected chromatographically after three hours' boiling with dilute sulphuric acid. Under such conditions the spectrum of the reaction mixture remains that of a chrysoaphin, despite the fact that even a small quantity of an erythroaphin could be detected, if present, by its characteristic absorption in the 500—600 mμ region.

In marked contrast to these observations, compound A on treatment with zinc and acetic acid is readily converted into a new erythroaphin derivative, compound B, $C_{30}H_{22}O_9$. This is isomeric with the hydroxyerythroaphin-*fb* (VII; R = OH) produced by periodate oxidation or *via* Thiele acetoxylation [4] of erythroaphin-*fb* (VII; R = H) and it shows absorption in the infrared at 3300 $cm.^{-1}$, corresponding to a non-bonded hydroxyl group.

[4] B. R. Brown, A. W. Johnson, S. F. MacDonald, J. R. Quayle, and A. R. Todd, *J.*, 1952, 4928.

There are, however, a number of differences between the two isomeric hydroxyerythroaphins. Their visible spectra (Figure), though related to one another, indicate some chromophoric difference between them. In accord with this, compound B is deep blue in alkaline solution whereas both hydroxyerythroaphin-*fb* and the erythroaphins themselves give green solutions. Finally, compound B is clearly the more stable towards reducing agents since it is formed under conditions in which hydroxyerythroaphin-*fb* would be reduced to the erythroaphin.[4] When treated with acetic anhydride in the presence of sodium acetate, conditions which do not acetylate the *peri*-hydroxyl groups in erythroaphins, it yields a product whose visible spectrum is virtually identical with that of an erythroaphin and whose infrared spectrum lacks the band at 3300 cm.$^{-1}$ but contains one at 1775 cm.$^{-1}$ characteristic of a phenolic acetate. When treated with alkali, this acetylated product is reconverted into compound B. These observations strongly suggest that the chromophore of compound B is 4,9-dihydroxyperylene-3,10-quinone, substituted by an acylable hydroxyl group. Since, in addition, there is no obvious reason why the non-aromatic side chains in chrysoaphin-*sl*-1 should have been cleaved on periodate oxidation (those of



Ultraviolet absorption of (A) 2-hydroxyerythroaphin-*sl* (II; R = OH) and (B) hydroxyerythroaphin-*fb* (VII; R = OH) in chloroform

erythroaphins are not) it is assumed that they are intact in compound B, which is therefore formulated as 2-hydroxyerythroaphin-*sl* (II; R = OH), numbering the perylene nucleus in the usual way. Compound B is the first erythroaphin derivative obtained, other than the halogeno-derivatives,[5] bearing a nuclear substituent. Direct hydroxylation of erythroaphin, as has already been noted, leads to substitution in the side-chain. The reasons for preferring attachment of the hydroxyl group in the 2-position shown in (II; R = OH) and not, for example, to the 8-position diagonally opposite, and for retention of the *sl* stereochemistry, will be discussed subsequently. Further confirmation of this structure follows from reductive acetylation, which readily yields 2-acetoxytetra-acetyldihydroerythroaphin-*sl* (VIII; R = OAc). This compound could also be prepared directly from compound A by treatment with zinc, acetic anhydride, and sodium acetate. Its visible spectrum is virtually identical with that of tetra-acetyldihydroerythroaphin-*sl* (VIII; R = H), *i.e.*, both contain essentially a perylene system substituted by two ethereal oxygens. Its infrared spectrum shows absorption due to aromatic esters (1779 cm.$^{-1}$) and none due to aliphatic. When treated with methanolic sodium hydroxide this pentaacetate regenerates 2-hydroxyerythroaphin-*sl* (II; R = OH).

[5] D. W. Cameron, R. I. T. Cromartie, Y. K. Hamied, P. M. Scott, and Lord Todd, *J.*, 1964, 62.

The structure of compound A cannot be directly inferred from the production of 2-hydroxyerythroaphin-*sl* (II; R = OH) by treatment with zinc and acetic acid. Useful information can, however, be obtained from catalytic reduction. Hydrogenation of compound A in acetic acid proceeded with uptake of 2·6 mols. hydrogen but no homogeneous product could be isolated from the reaction. (Under the same conditions 2-hydroxyerythroaphin-*sl* itself took up 1·8 mols. and also yielded largely unidentified products.) However, catalytic reductive acetylation of compound A in acetic anhydride proceeded much more smoothly with uptake of 1·0 mol. hydrogen and formation of a new substance, compound C, $C_{32}H_{26}O_{11}$, shown by its nuclear magnetic resonance (n.m.r.) spectrum to be a mono-acetate (3 protons at 7·55 τ). Similar reductive acetylation of chrysoaphin-*sl*-1 itself gave only starting material, *i.e.*, neither *peri* nor hemiketal hydroxyl groups of the chrysoaphin system are acetylated under these conditions.

The chromophore of compound C is undoubtedly that of a chrysoaphin. Like chrysoaphin but unlike compound A it is readily decomposed by acid or alkali. On treatment with the latter, it yields 2-hydroxyerythroaphin-*sl* (II; R = OH); with dilute mineral acid it is first converted into the corresponding acetate (II; R = OAc) and thence, on more vigorous treatment into the hydroxy-compound. The alkaline reaction also may involve the acetate as intermediate, for it is accompanied by a smooth colour change from pink through green and finally to blue. These colours correspond to the anions of a chrysoaphin system, an erythroaphin system, and 2-hydroxyerythroaphin-*sl* (II; R = OH) respectively. These observations can be accommodated only by two structures for compound C, *viz.* (III; R = OAc; R′, R″ = H) and (III; R, R′ = H; R″ = OAc). The latter can be eliminated by n.m.r. considerations [other than the penta-acetate (VIII; R = OAc), compound C is the only product described in this work, for which a clear n.m.r. spectrum could be obtained. Apart from the protons of the acetate group, already described, the spectrum showed two singlets, each due to one proton, at 3·15 and 3·85 τ and assigned to protons R″ and R′ in structure (III; R = OAc; R′, R″ = H). The methyl region, as expected, consisted of four overlapping doublets (8·3, 8·3, 8·5, 8·8 τ, J ~6 cycles per sec.) as for erythroaphin-*sl*,[6] while the region *ca.* 5·0 τ, though not resolvable, was similar to that in the spectrum of erythroaphin-*sl*. Compound C is therefore an acetoxychrysoaphin-*sl*-1. It absorbed in the infrared at 1772 and 1665 cm.$^{-1}$. On the basis of formula (III; R = OAc; R′, R″ = H) these are assigned to carbonyl groups of the acetate and of the neighbouring keto-group respectively. Their high frequency is presumably due to interaction between the two carbonyl groups such as has been observed to cause similar frequency shifts in other α-acyloxy-ketones.[7]

We may now consider the structure of compound A itself. It has so far been established that this substance, though a chrysoaphin, does not undergo dehydration to an erythroaphin but that on reductive acetylation, a process involving uptake of 1 mol. hydrogen it is converted into acetoxychrysoaphin-*sl*-1 (III; R = OAc; R′, R″ = H). This means that compound A is two oxidation levels higher than chrysoaphin. Reductive acetylation is accompanied by the loss of one oxygen atom. This most probably is one of the two introduced by periodate, since it has already been noted that chrysoaphin-*sl*-1 itself is unaffected by similar reductive acetylation. The second oxygen introduced by periodate is obviously that of the acetylated hydroxyl group in acetoxychrysoaphin-*sl*-1 (III; R = OAc; R′, R″ = H). To accommodate these observations the most plausible formulation for compound A is the dihydroxychrysoaphin-*sl*-1 (III; R, R′ = OH; R″ = H). This cannot be converted into an erythroaphin unless one of the introduced hydroxyl groups is first reductively removed. The existence of a stable *gem*-diol in such a system is unexceptional. It is analogous to cyclohexane-1,2,3-trione dihydrate, which is, in

[6] D. W. Cameron, R. I. T. Cromartie, Y. K. Hamied, P. M. Scott, N. Sheppard, and Lord Todd, *J.*, 1964, 90.

[7] L. J. Bellamy, " The Infra-red Spectra of Complex Molecules," Methuen, London, 2nd edn., 1958, p. 146.

fact, the form in which this substance is obtained,[8] the free trione never having been isolated. Both dihydroxychrysoaphin-*sl*-1 and its bromo-derivative, now formulated as (III; R, R′ = OH; R″ = Br) absorb in the infrared at 1660 cm.$^{-1}$, 22 cm.$^{-1}$ higher than chrysoaphin.[2] This is presumably due to the keto-group influenced by the *gem*-diol, *e.g.*, the two peaks in the carbonyl region of ninhydrin have frequencies 8 and 14 cm.$^{-1}$ higher than those of 2,2-dimethylindane-1,3-dione.[9]

The structures assigned to dihydroxychrysoaphin-*sl*-1 and its derivatives, while explaining most of the chemistry so far described, nevertheless have limitations, some of which are discussed below. Our conclusions therefore must be regarded as provisional. We should have preferred to examine this problem more extensively but our stocks of chrysoaphins-*sl* are low and little more appears likely to be obtainable in the near future. Our only source of material, the willow aphid *Tuberolachnus salignus* suffered heavily during the winter of 1961—1962 and has not been available in the Cambridge area since then. Further, the chemical complexity of these systems means that adequate model compounds are virtually as inaccessible synthetically as the natural substances themselves.

The introduction of two hydroxyl groups into chrysoaphin-*sl*-1 on periodate oxidation is clearly a radical process rather than the more usual glycol fission reaction. Similar processes are presumably involved in the oxidation of aldol [10] and β-diketone [11] systems. In agreement with this, the use of purified dioxan as solvent for the oxidation gave a very poor yield of dihydroxychrysoaphin-*sl*-1. The addition of benzoyl peroxide (or, alternatively, use of unpurified dioxan) is necessary for the production of reasonable yields. Hydrogen peroxide, on the other hand, is ineffective, as is benzoyl peroxide in the absence of periodate. Other oxidising agents such as lead tetra-acetate or permanganate cause decomposition of chrysoaphins while manganese dioxide is without action. It is interesting to note that in the periodate oxidation, attempts to stop the process at the monohydroxychrysoaphin stage were unsuccessful. Evidently the introduction of the second hydroxyl group is faster than that of the first. Dihydroxychrysoaphin-*sl*-1 (III; R, R′ = OH; R″ = H) is relatively stable to periodate, decomposing slowly during a period of hours to unidentifiable products. Why this system does not undergo glycol, acyloin, or dione cleavage is not obvious. Examples of such systems [12] that are indeed relatively stable to periodate are known but no adequate model to compare with dihydroxychrysoaphin-*sl*-1 appears to be available. The fact that the transition state for periodate cleavage of dihydroxychrysoaphin-*sl*-1 would probably be sterically crowded might also be a stabilising factor.

It remains now to assign structures to chrysoaphins-*sl*-1 and -*sl*-2 and, in so doing, to justify the assumptions, made earlier, that in 2-hydroxyerythroaphin-*sl* (II; R = OH) the hydroxyl group was substituent to the position indicated and that the *sl* stereochemistry was indeed retained. With regard to the latter, we consider the possibility of an *sl* ⟶ *fb* epimerisation during oxidation as small since hydroxylation of chrysoaphin-*sl*-1, unlike that of erythroaphin-*sl*, does not occur at the epimeric centre. Had the former assumption not been made, the structure of dihydroxychrysoaphin-*sl*-1 would have been deduced as either (III; R, R′ = OH; R″ = H) or (IV; R, R′ = OH). We can exclude the latter because, as has been noted, chrysoaphins-*fb* and -*sl*-2, in contrast to -*sl*-1, are not converted into dihydroxychrysoaphins on oxidation. This specificity towards oxidation is much more likely to be determined by the stereochemistry of the hemiketal-containing side chain in chrysoaphin-*sl*-1 than by that on the opposite side of the aromatic system, since it is on the former side that hydroxylation actually occurs. This implies that since chrysoaphins-*fb* and -*sl*-2 behave similarly towards periodate, the stereochemistry of their

[8] B. Pecherer, L. M. Jampolsky, and H. M. Wuest, *J. Amer. Chem. Soc.*, 1948, **70**, 2587.
[9] D. G. O'Sullivan, *J.*, 1960, 3278.
[10] A. J. Birch, D. W. Cameron, Y. Harada, and R. W. Rickards, *J.*, 1961, 889.
[11] M. L. Wolfrom and J. M. Bobbitt, *J. Amer. Chem. Soc.*, 1956, **78**, 2489.
[12] *E.g.*, E. Adler, R. Magnusson, and B. Berggren, *Acta Chem. Scand.*, 1960, **14**, 539; V. C. Bulgrin and G. Dahlgren, *J. Amer. Chem. Soc.*, 1958, **80**, 3883.

hemiketal-containing side chains must be the same. Since that of the former (VI) is known the structure of the latter must be (IV; R, R′ = H) and chrysoaphin-*sl*-1, accordingly (III; R, R′, R″ = H). Similarly, dihydroxychrysoaphin-*sl*-1 and its degradation products must have structures based on (III) and not on (IV). Why the stereochemistry of the former leads to reactivity towards periodate, while that of the latter does not, is not obvious. It clearly must depend both on the mechanism of oxidation and on the conformation of non-aromatic rings in chrysoaphins, neither of which is known in detail. As would be expected, n.m.r. spectroscopy has shown considerable conformational differences between erythroaphins-*fb* and -*sl*,[13] but chrysoaphins, being less soluble are not amenable to study by this technique.

Experimental

Unless otherwise stated, ultraviolet and visible spectra were measured in chloroform solution, and infrared spectra in Nujol mulls.

Paper Chromatography.—Methods were as previously described [2] with mixtures of chloroform, light petroleum (b. p. 120°), and water: solvent *A*, 4 : 1 : 1; solvent *B*, 1 : 1 : 1.

Oxidation of Chrysoaphin-sl-1.—(*a*) Chrysoaphin-*sl*-1 (500 mg.) in unpurified commercial dioxan (100 ml.), was added during 10 min. to a refluxing solution of sodium metaperiodate (500 mg.) in dioxan (50 ml.)–water (100 ml.). Boiling was continued for 1 hr., the mixture diluted with water, and then extracted with ether (6 × 150 ml.). The extract was washed with water and with sodium hydrogen carbonate, dried, and evaporated to yield a dark red gum, which was dissolved in the minimum volume of chloroform and chromatographed on silicic acid (Mallinckrodt 2847; 130 g.). Elution with chloroform gave the following fractions: (i) erythroaphin-*sl* (30 mg.), R_F in solvent *B* identical with that of authentic material; (ii) a mixed fraction of erythroaphin-*sl*, hydroxyerythroaphin-*fb*, and unidentified materials; (iii) hydroxyerythroaphin-*fb* (90 mg.). After recrystallisation from chloroform–ethanol it had R_F in solvent *A* 0·66 and infrared absorption identical with that of authentic material; (iv) dihydroxychrysoaphin-*sl*-1 (90 mg.), see (*b*) below; (v) two red bands both yielding material having R_F in solvent *A* 0·0, $\lambda_{max.}$ 458, 536, 580, $\lambda_{infl.}$ 410, 435, 480, 600 mμ and $\lambda_{max.}$ 430, 456, 533, 575, 600, $\lambda_{infl.}$ 410, 485 mμ respectively. These are presumably more highly hydroxylated erythroaphins.

(*b*) The procedure in (*a*) was repeated with purified dioxan containing benzoyl peroxide (100 mg.) and refluxing the mixture for 1·5 hr. Chromatography as above yielded *dihydroxychrysoaphin*-sl-1 (250 mg.) which after recrystallisation from acetone had R_F in solvent *A* 0·36 (Found, on a sample dried at 60° over P_2O_5: C, 63·8; H, 4·7. $C_{30}H_{24}O_{11}$ requires C, 64·1; H, 4·4%); $\lambda_{max.}$ 268, 381, 403, 456, 487 mμ (log ε 4·66, 4·39, 4·52, 4·20, 4·23); $\nu_{max.}$ 3300, 1660, 1645, 1613, 1590, 1277, 1244, 1205, 1171, 1153, 1130, 1107, 1093, 1074, 1045, 1022, 1009, 995, 976, 960, 939, 917, 894, 850, 823, 807, 787, 755, 694, 667 cm.$^{-1}$; $\nu_{max.}$ in CCl_4 1655 (sh.) 1642, 1612 cm.$^{-1}$.

Periodate Oxidations.—Samples of the following compounds (1—10 mg.) were boiled in aqueous dioxan (10 ml.) containing their own weight of sodium metaperiodate and one fifth their weight of dibenzoyl peroxide. The reaction mixture was examined at intervals by paper chromatography. (*a*) Chrysoaphin-*fb*: after 90 min. erythroaphin-*fb*, starting material, and red material of R_F 0 were detected. (*b*) Chrysoaphin-*sl*-2: as above; unchanged starting material could be detected even after 6 hr. (*c*) Dihydroxychrysoaphin-*sl*-1: after 1 hr. starting material together with yellow material of R_F 0 were detected. (*d*) Erythroaphins-*fb* and -*sl*: both behaved similarly and after 11 hr. were largely converted into a red product of R_F 0 together with a little hydroxyerythroaphin-*fb*. (*e*) Hydroxyerythroaphin-*fb*: slowly converted into products of R_F 0·0 and 0·2 in solvent *A*; some unchanged starting material remained after 6 hr.

Bromodihydroxychrysoaphin-sl-1.—Bromine (0·1 ml.) was added to a suspension of dihydroxychrysoaphin-*sl*-1 (60 mg.) in glacial acetic acid (3·0 ml.) and the mixture shaken for 2 hr. Solution was complete after 30 min. and after 1 hr. a precipitate of *bromodihydroxychrysoaphin*-sl-1 began to form. This was filtered off at the end of the reaction and recrystallised from ether–petroleum (b. p. 40—60°) as orange needles (40 mg.) (Found: C, 56·5; H, 4·0; Br, 11·9. $C_{30}H_{23}BrO_{11}$ requires C, 56·3; H, 3·6; Br, 12·5%); $\lambda_{max.}$ 270, 390, 410, 457, 487 mμ (log ε 4·77, 4·50, 4·62, 4·22, 4·32); $\nu_{max.}$ 3350, 1660, 1650, 1610, 1572 cm.$^{-1}$.

Acetoxychrysoaphin-sl-1.—A mixture of platinum oxide (10 mg.), anhydrous sodium acetate

[13] D. W. Cameron, D. G. I. Kingston, N. Sheppard, and Lord Todd, *J.*, 1964, 98.

(10 mg.), and acetic anhydride (4 ml.) was hydrogenated. Dihydroxychrysoaphin-*sl*-1 (40 mg.) was added and hydrogenation continued. After 1 hr. the pigment went into solution and after a further 30 min. a yellow solid precipitated. It was filtered off and extracted into ether from a Soxhlet thimble. Addition of petroleum (b. p. 60—80°) and evaporation of the ether on a water-bath caused crystallisation of *acetoxychrysoaphin*-sl-1 (25 mg.) as yellow needles (Found: C, 64·8; H, 4·7. $C_{32}H_{26}O_{11}$ requires C, 64·8; H, 4·5%); $\lambda_{max.}$ 268, 382, 402, 460, 486 mμ (log ε 4·69, 4·42, 4·54, 4·10, 4·20); $\nu_{max.}$ 3320, 1770, 1660, 1641, 1613, 1590, 1456, 1285, 1237, 1210, 1190, 1173, 1157, 1143, 1106, 1082, 1074, 1061, 1038, 1010, 975, 955, 948, 922, 887, 852, 833, 815, 774, 759, 752, 715, 696, 687 cm.$^{-1}$; R_F in solvent *A* 0·93.

2-*Hydroxyerythroaphin*-sl.—(*a*) Dihydroxychrysoaphin-*sl*-1 (55 mg.) was warmed on a water-bath with zinc dust (100 mg.) and acetic acid (10 ml.) for 15 min., during which time the solution became red. The mixture was poured into water, extracted with ether, and the extract washed, dried, and solvent evaporated. The product was chromatographed on silicic acid (Mallinckrodt 2847, 40 g.) in chloroform. Recrystallisation of the main fraction from chloroform gave 2-*hydroxyerythroaphin*-sl as a dark red solid (Found: C, 67·9; H, 4·5. $C_{30}H_{22}O_9$ requires C, 68·4; H, 4·2%); $\lambda_{max.}$ 264, 349, 442, 516, 561 mμ (log ε, 4·63, 3·62, 4·33, 4·31, 4·22); $\lambda_{infl.}$ 420, 490 mμ (log ε 4·17, 4·26); $\lambda_{max.}$ in dioxan 226, 264, 348, 439, 512, 550 mμ (log ε 4·69, 4·63, 3·67, 4·32, 4·33, 4·21), $\lambda_{infl.}$ 418, 488, 585 mμ (log ε 4·18, 4·25, 3·74); $\lambda_{max.}$ in aqueous acetone (1 : 1) containing a few drops of aqueous sodium hydroxide 418, 584 mμ, $\lambda_{infl.}$ 442 mμ; $\nu_{max.}$ 3300, 1628, 1570, 1335, 1305, 1275, 1242, 1205, 1174, 1155, 1115, 1080, 1050, 1000, 975, 947, 850, 830, 739 cm.$^{-1}$; R_F in solvent *B* 0·65.

(*b*) A solution of acetoxychrysoaphin-*sl*-1 (25 mg.) in acetone (40 ml.) was treated with aqueous sodium hydroxide (10 ml. of 0·1N) and the mixture warmed at 50° for 15 min. The initial bright red colour of the solution rapidly became green and then deep blue. When heating was complete the mixture was acidified (15 ml. of 0·1N-hydrochloric acid) and extracted with ether to yield 2-hydroxyerythroaphin-*sl* which was triturated with ice-cold ether (20 ml.) and recrystallised from chloroform–ethanol. The product (10 mg.) was identical with that from (*a*) in ultraviolet, visible, and infrared spectra.

2-*Acetoxytetra-acetyldihydroerythroaphin*-sl.—(*a*) 2-Hydroxyerythroaphin-*sl* (35 mg.) was reductively acetylated by the procedure previously described for dichloroerythroaphin-*sl*.[14] The crude product was chromatographed in benzene on a column of silica gel. Elution with benzene–ethyl acetate (9 : 1) gave a yellow solution having a green fluorescence. Evaporation to dryness followed by crystallisation of the resulting orange gum from ether–light petroleum gave 2-*acetoxytetra-acetyldihydroerythroaphin*-sl (31 mg.) as a yellow solid (Found: C, 64·9; H, 4·7. $C_{40}H_{34}O_{14}$ requires C, 65·1; H, 4·6%); $\lambda_{max.}$ 278, 357, 408, 434, 463, 496 mμ (log ε 4·53, 3·34, 3·62, 3·93, 4·25, 4·35); $\nu_{max.}$ 1779, 1604, 1581, 1332, 1264, 1194, 1140, 1112, 1074, 1037, 1027, 875, 795 cm.$^{-1}$.

(*b*) A mixture of dioxychrysoaphin-*sl*-1 (55 mg.) and fused sodium acetate (30 mg.) was suspended in acetic anhydride (10 ml.) and zinc dust (2 g.) added. It was then shaken at room temperature for 5 hr., filtered, the filtrate poured into water, and the mixture extracted with ether. The extract was washed, dried, and evaporated and the product worked up as in (*a*) to yield 2-acetoxytetra-acetyldihydroerythroaphin-*sl* (30 mg.), identical in ultraviolet, visible, and infrared spectra with authentic material.

Treatment of this compound (13 mg.) in chloroform (25 ml.) with methanol (30 ml.) and aqueous sodium hydroxide (4 ml. of 10%), shaking the mixture for one minute, acidifying with dilute hydrochloric acid, and separating the chloroform layer yielded 2-hydroxyerythroaphin-*sl* (4 mg.). Its ultraviolet, visible, and infrared spectra were identical with those of authentic material.

2-*Acetoxyerythroaphin*-sl.—(*a*) A solution of acetoxychrysoaphin *sl*-1 (2 mg.) in chloroform (4 ml.) was treated with ethanol containing concentrated hydrochloric acid (1 ml.) at 50° for 5 min. The yellow solution turned red. Dilution with water and extraction with ether yielded 2-acetoxyerythroaphin-*sl* having R_F in solvent *B* 0·95; $\lambda_{max.}$ in ether 450, 522, 564 mμ, $\lambda_{infl.}$ 405, 430, 482, 590 mμ; $\nu_{max.}$ 1775, 1625, 1571 cm.$^{-1}$.

Treatment of this substance with ethanolic hydrochloric acid at 100° for 10 min. yielded 2-hydroxyerythroaphin-*sl*, having R_F value and ultraviolet and visible spectra identical with those of authentic material.

[14] D. W. Cameron, R. I. T. Cromartie, Y. K. Hamied, B. S. Joshi, P. M. Scott, and Lord Todd, *J.*, 1964, 72.

(*b*) A suspension of 2-hydroxyerythroaphin-*sl* (2 mg.) and sodium acetate (10 mg.) in acetic anhydride (5 ml.) was shaken for 3 hr. and then filtered. The filtrate was decomposed with aqueous acetic acid, extracted with ether, and the extract washed with water and aqueous sodium hydrogen carbonate. The product had R_F value, ultraviolet, and visible spectra identical with that from (*a*).

(*c*) A mixture of acetoxychrysoaphin-*sl*-1 (25 mg.) and acetic anhydride (2 ml.) was warmed at 70—80° for 2 hr. giving a deep red solution which was then poured into ice-cold aqueous sodium acetate. Extraction with ether followed by washing of the extract with aqueous sodium hydrogen carbonate gave a red solid which was chromatographed in benzene on silica gel. Benzene eluted 2-acetoxyerythroaphin-*sl* (6 mg.) obtained as a red black granular solid after recrystallisation from carbon tetrachloride, and was identical in R_F value, ultraviolet, visible, and infrared spectra with the product from (*a*).

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