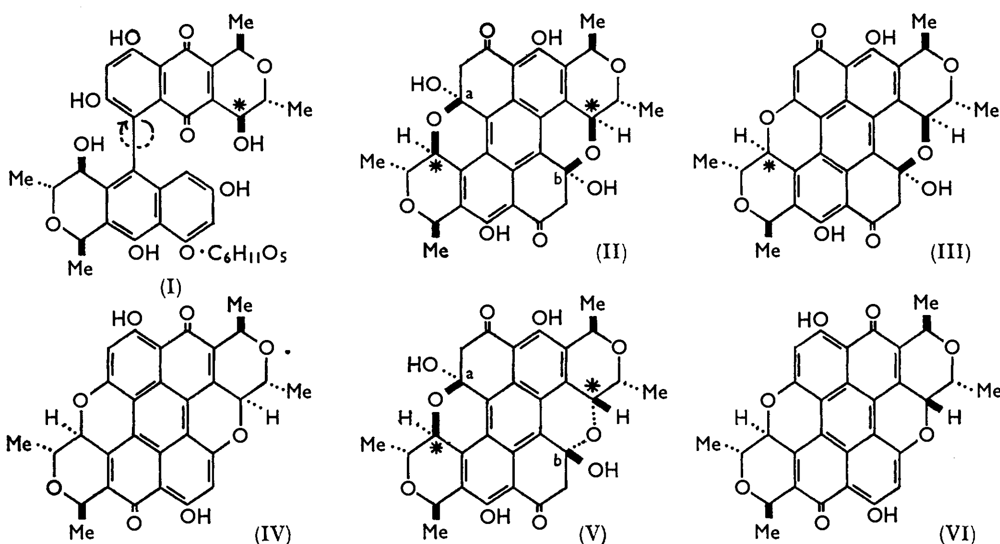
13. *Colouring Matters of the Aphididæ. Part XX.*[1] *The Structure of the Xanthoaphins and Chrysoaphins.*

By A. CALDERBANK, D. W. CAMERON, R. I. T. CROMARTIE, Y. K. HAMIED, E. HASLAM, D. G. I. KINGSTON, LORD TODD, and J. C. WATKINS.

Structures are assigned to the xantho- and chryso-aphins. An aphin series, protoaphin-*fb* (I) ⟶ xanthoaphin-*fb* (II) ⟶ chrysoaphin-*fb* (III) ⟶ erythroaphin-*fb* (IV), structurally and stereochemically complete except for minor details, is shown and possible mechanisms of pigment interconversions are discussed. The production of two chrysoaphins-*sl* is also explained. Vigorous chlorination of erythroaphin-*fb* yields a complex chlorinated product, spectrally similar to a xanthoaphin but, unlike the latter, stable enough to be identified as an anthracene derivative. This confirms the anthracene formula assigned to the xanthoaphins.

IN earlier papers of this series the structure of protoaphin-*fb* has been shown to be (I) and that of protoaphin-*sl* to be (I; epimeric at C*),[2] while erythroaphin-*fb* and -*sl* have respectively structures (IV) and (VI),[3] the absolute stereochemistry being in each case as shown in the formulæ. The water-soluble protoaphins which occur in the hæmolymph of living aphids are attacked, after the death of the insects, by a specific enzyme system [4,5] present in them, and yield successively the xanthoaphins, chrysoaphins, and erythro-



aphins belonging to the two aphin series (*fb* and *sl*). The same changes can be initiated by pigment-free enzyme extracts prepared from fresh insects,[6] and whether the enzyme reaction is carried out *in vitro* or *in vivo* the xanthoaphins, yellow fat-soluble, unstable substances, are obtained as the first isolable products. The chrysoaphins which are orange are somewhat more stable and can be obtained from the xanthoaphins by mild acid or alkaline treatment and are themselves converted by more vigorous treatment into the erythroaphins which are the stable end products of the aphin series. It has been

[1] Part XIX, Cameron, Cromartie, Hamied, Joshi, Scott, and Todd, preceding paper.
[2] Part XVII, Cameron, Cromartie, Kingston, and Todd, *J.*, 1964, 51.
[3] Part XVIII, Cameron, Cromartie, Hamied, Scott, and Todd, *J.*, 1964, 62.
[4] Human, Johnson, MacDonald, and Todd, *J.*, 1950, 477.
[5] Duewell, Johnson, MacDonald, and Todd, *J.*, 1950, 485.
[6] Brown, Ekstrand, Johnson, MacDonald, and Todd, *J.*, 1952, 4925.

established spectroscopically that smooth conversion of each of these pigments into the succeeding member of the series occurs and that no other intermediates can be detected. It follows that only the structures of the xanthoaphins and chrysoaphins remain to be determined to complete our survey of the aphin pigments and their interconversions. It will be shown herein that in the *fb* series these are represented by (II) and (III), respectively, structures which it is convenient to introduce at this stage in order to facilitate discussion and also to illustrate, for the first time, the structural implications of a complete aphin series. The conversions protoaphin-*fb* ⟶ xanthoaphin-*fb* ⟶ chrysoaphin-*fb* ⟶ erythroaphin-*fb* can be represented by (I) ⟶ (II) ⟶ (III) ⟶ (IV). A full discussion of the pathway is given later, together with details of the *sl* series for which similar formulæ can be written.

Paper-chromatographic [7] studies have shown that the relation between the xanthoaphins and chrysoaphins is more complex than had earlier been assumed. Protoaphin-*fb* is converted into one, and only one, xanthoaphin-*fb* and this in turn into one chrysoaphin-*fb*. In the *sl* series, however, although only one xanthoaphin-*sl* is produced, the latter yields two isomeric chrysoaphins each convertible into one and the same erythroaphin-*sl*. For convenience we describe these isomers as chrysoaphin-*sl*-1 and chrysoaphin-*sl*-2, numbering them in order of decreasing R_F values on paper chromatograms. From mixed chrysoaphins-*sl* the *sl*-1 isomer can be isolated by direct crystallisation. The *sl*-2 isomer is obtained by chromatography after treatment of the mixture with sodium metaperiodate which converts chrysoaphin-*sl*-1 into hydroxylated products. The nature of this remarkable reaction with periodate, which is not shown by the other chrysoaphins, is not yet fully understood and experiments aimed at its elucidation are in progress. For the work described in this paper xanthoaphin-*sl* was prepared by enzyme treatment of isolated protoaphin-*sl in vitro* and it was converted with acid under the mildest possible conditions into the chrysoaphins. When the mixed aphins-*sl* produced by fermentation in insects stored in bulk were examined by paper chromatography, small amounts of additional components comparable in R_F with xanthoaphin and chrysoaphin were observed. These were less stable than the true aphins, could not be isolated in a pure state, and have not been examined in detail; whether they represent minor related pigments or merely products of aerobic decomposition in the stored insects is not at present known.

In the light of our more recent work the molecular formulæ previously put forward tentatively for the xanthoaphins and chrysoaphins [4,5] must be revised. Elementary analyses of chrysoaphin-*fb*, -*sl*-1, and -*sl*-2 indicate a formula $C_{30}H_{24}O_9$ in each case. Conversion of chrysoaphin into erythroaphin thus corresponds to a loss of 1 H_2O. The chrysoaphins, like the erythoaphins, show the characteristic quinonoid behaviour of reversible reduction by sodium dithionite, but are more acidic and in dimethylformamide solution can be titrated with tetra-n-butylammonium hydroxide as monobasic acids of equivalent weight 520, in reasonable agreement with the proposed molecular formula. Further addition of alkali converts them into erythroaphins and a second break in the titration curve can then be observed corresponding to the second dissociation of the latter. Although the chrysoaphins are considerably more stable than the xanthoaphins, no simple derivatives of them have been obtained; thus attempted acetylation or reductive acetylation by standard procedures yields only the corresponding acetylated erythroaphins. It has proved equally impossible to prepare simple derivatives from the xanthoaphins, the elementary analysis of which is rendered difficult by their extreme lability and their tendency to retain solvent of crystallisation. However, by careful purification, samples of xanthoaphin-*fb* and -*sl* have been obtained which give satisfactory analyses for $C_{30}H_{26}O_{10}$, and therefore lose 2 H_2O in passing into the erythroaphins. Unlike any other aphins, the xanthoaphins do not exhibit the redox behaviour characteristic of quinones.

The electronic absorption spectra [4,5] of the fluorescent aphins consist of a series of

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

rather sharp bands and they are virtually identical for isomeric pigments, but the maxima are displaced progressively to longer wavelengths on passing along the series xanthoaphin ⟶ chrysoaphin ⟶ erythroaphin. Considered in conjunction with the results of elementary analysis of the pigments this suggests that the series corresponds to a progressive aromatisation of the central part of the molecule by elimination of water. Support for this suggestion comes from the presence in the infrared spectra [8] of the xanthoaphins and chrysoaphins of a strong band in the hydroxyl stretching region near 3300 cm.$^{-1}$ characteristic of free hydroxyl groups, whereas in the erythroaphins there is only a weak band near 2700 cm.$^{-1}$ corresponding to strongly chelated hydroxyl groups. That this difference is due to additional hydroxyl groups and not to suppression of chelation is clear from the position of the carbonyl band at 1625 cm.$^{-1}$ in the xanthoaphins, which is too low for a non-chelated carbonyl group. The corresponding band in chrysoaphin is broad and is partially split under high resolution into two bands with maxima at 1643 and 1633 cm.$^{-1}$, suggesting that the two carbonyl groups in the chrysoaphins differ in their environment. Both analytical and spectroscopic data thus support the view that chrysoaphin contains one, and xanthoaphin two, hydroxyl groups which are eliminated as water to form two of the aromatic double bonds of erythroaphin.

The fact that the elimination of these additional hydroxyl groups in the xanthoaphins and chrysoaphins is catalysed, not only by acids, but also by bases would be most readily explained if they were situated in the β-position with respect to carbonyl or potential carbonyl groups. Furthermore, the formation of only one chrysoaphin-*fb* from xanthoaphin-*fb* in contrast to the production of two different chrysoaphins from xanthoaphin-*sl* indicates that the two extra hydroxyl groups in xanthoaphin-*fb* are identically situated, so that elimination of either of them leads to one and the same chrysoaphin.

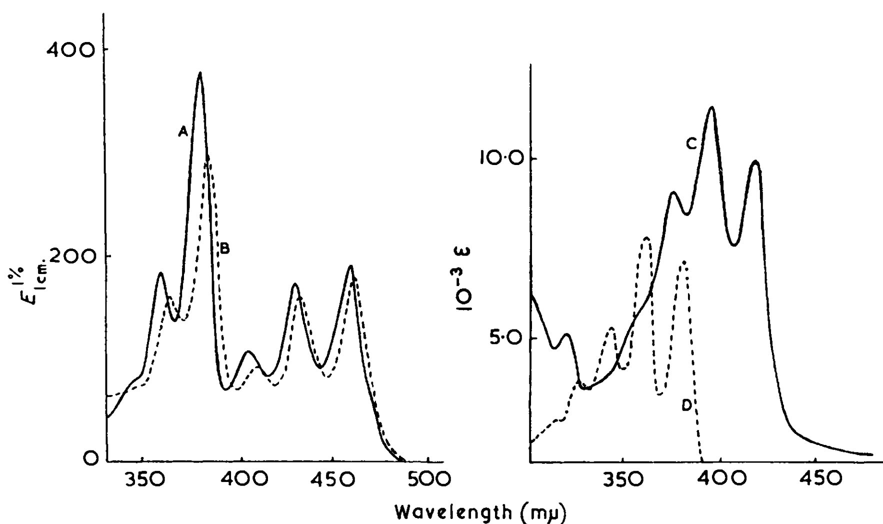
The above evidence is best accommodated by formulæ (II) and (V) for xanthoaphin-*fb* and -*sl*, respectively. The stereochemistry of the two new asymmetric carbons in each molecule, *viz.*, those bearing the hemiketal hydroxyl groups, is assigned from considerations of strain which would be expected to preclude the alternative configuration. The stereochemistry at these centres is seen, therefore, to be determined by the stereochemistry at the carbons marked with asterisks in (II) and (V), which is fixed. These structures contain no quinone function, in accordance with experimental observation. Formation of the chrysoaphin is envisaged as proceeding by a simple dehydration, so that chrysoaphin-*fb* becomes (III) (or a related tautomer) which now contains a redox system. Since in xanthoaphin-*fb* (II) the two hemiketal hydroxyl groups are equivalent, only one chrysoaphin-*fb* is possible, but in xanthoaphin-*sl* (V) dehydration can give rise to two, isomeric chrysoaphins depending on which of the two hemiketal hydroxyls is involved in the dehydration. It is not possible at present to define which of these two chrysoaphins corresponds to the *sl*-1 isomer and which to the *sl*-2, but it is evident that loss of a further mol. of water from either must lead to the same erythroaphin-*sl* (VI).

The light absorption [4,5] of the xanthoaphins is not inconsistent with their formulation as anthracene derivatives as in structures (II) and (V). The xanthoaphins themselves are far too unstable to permit their conversion into any substance with a single anthracene chromophoric system which could be recognised with certainty by comparison with known compounds, but this comparison has been made by an interesting indirect method. When erythroaphin-*fb* (IV) is treated for a short time with chlorine in hot nitrobenzene a beautifully crystalline yellow compound is obtained,* which from its light absorption (the Figure) evidently has the same chromophore as the xanthoaphins, but it does not share their instability. Its infrared spectrum shows a band at 1656 cm.$^{-1}$ in the carbonyl region consistent with a hydrogen-bonded (chelated) aromatic chloro-ketone and, like the xanthoaphins, a strong band near 3300 cm.$^{-1}$. Analysis of the compound and of its crystalline

* This reaction was first observed by Dr. J. R. Quayle.

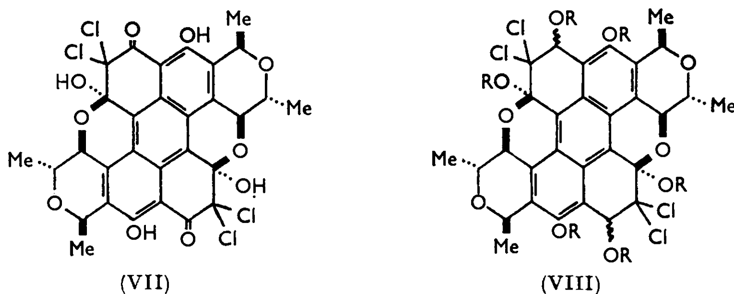
[8] Johnson, Quayle, Robinson, Sheppard, and Todd, *J.*, 1951, 2633.

tetra-acetyl derivative show it to have a formula $C_{30}H_{22}Cl_4O_{10}$ and we regard it as being most probably the tetrachloroxanthoaphin-*fb* (VII). This anomalous chlorination of erythroaphin-*fb* we interpret as involving the introduction of two chlorine atoms by substitution (as is observed on mild chlorination [9] of the erythroaphins), followed by addition of the elements of hypochlorous acid across two double bonds in the erythroaphin nucleus, the necessary water being presumably derived from traces present in the nitrobenzene used as solvent. Since all four *peri*-oxygen groups are effectively equivalent



Ultraviolet absorption of (A) xanthoaphin, (B) tetrachloroxanthoaphin-*fb*, (C) hexa-acetyltetrachlorotetrahydroxanthoaphin-*fb*, and (D) anthracene, in $CHCl_3$.

because of strong hydrogen-bonding, the initial attack of Cl^+ (or its equivalent) would be expected to occur on the α-carbon atoms to give carbonium ions which could then take up a nucleophilic group in the β-position. Addition of hypochlorous acid to simpler quinones has been observed previously, *e.g.*, in the naphthaquinone series.[10] The presence of both phenolic and alcoholic hydroxyl groups in tetrachloroxanthoaphin-*fb* (VII) is confirmed by the infrared spectrum of its tetra-acetyl derivative which has ester bands at 1779



(aromatic) and 1750 cm.$^{-1}$ (aliphatic). The remaining carbonyl band in the spectrum (1717 cm.$^{-1}$) is consistent with a chlorinated non-bonded aromatic ketone. Elimination of water from (VII) to yield a related erythoaphin is, of course, impossible.

[9] Brown, Johnson, MacDonald, Quayle, and Todd, *J.*, 1952, 4928.
[10] Shvetsov and Shemyakin, *Zhur. obshchei Khim.*, 1949, **19**, 480.

Similar chlorination of dibromoerythroaphin-*fb* [9] yielded the same tetrachloroxanthoaphin-*fb* (VII) with displacement of the two bromine atoms. It was also obtained from di-iodoerythroaphin,[3] but in this case it could not be purified and its presence was shown spectroscopically and by paper chromatography. A similar product was also obtained from erythroaphin-*sl*, but it was amorphous and could not be purified. Further support for this formulation of the chlorination product as (VII) was obtained from reduction experiments. Treatment of compound (VII) with zinc and acetic acid yielded dichloroerythroaphin-*fb* (the halogen atoms in the dichloroerythroaphins are stable to reduction, in contrast to bromine in the analogous dibromo-derivatives [1]). Catalytic hydrogenation of compound (VII) in the presence of sodium acetate gave a product whose spectrum was that of a chrysoaphin derivative. This product was not analysed, but treatment with formic acid or alkali converted it into dichloroerythroaphin-*fb*. [When the above hydrogenation of compound (VII) was carried out in the absence of sodium acetate, no well-defined product could be isolated.] Similarly catalytic reductive acetylation yielded tetra-acetyldichlorodihydroerythroaphin-*fb*. These reductions are all readily explicable on the basis of formula (VII).

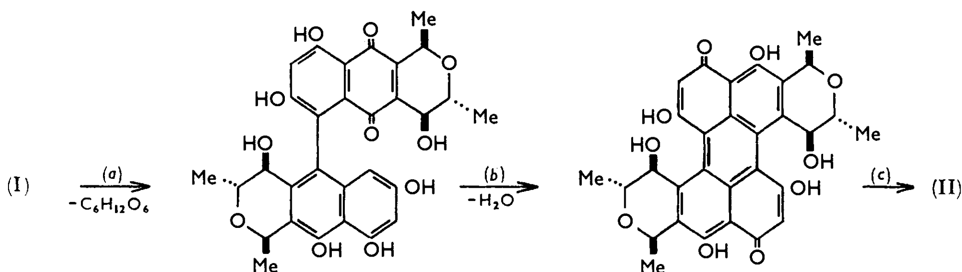
In order to identify the aromatic nucleus in compound (VII) as anthracene a variant of the spectroscopic method of Brockmann and Budde [11] was used. Reductive acetylation by the usual methods could not be employed as these caused removal of halogen and subsequent decomposition of the type mentioned above. The carbonyl groups were, however, smoothly reduced with sodium borohydride in methanol, giving an amorphous hydroxy-compound (VIII; R = H) which yielded a crystalline hexa-acetate (VIII; R = Ac). The ultraviolet absorption spectrum of this acetate shows a strong resemblance to that of anthracene especially in the fine structure of the long-wavelength band (the Figure); it shows of course a considerable bathochromic shift (35—40 mμ) and an increased intensity as compared with anthracene, but this is accommodated by the combined effects of the several alkyl and acetoxyl groups. This spectroscopic comparison strongly supports our view that the chlorination product and, by inference, the xanthoaphins contain an anthracene nucleus as required by structures (II) and (V).

It remains now to consider the chemistry involved in the protoaphin ⟶ xanthoaphin conversion. The complexity of this process, despite the mildness of the conditions under which it is carried out, has been recognised for many years and can be illustrated by some simple considerations. For example, three of the most characteristic functions in the protoaphins, which can be detected by cursory examination, *viz.*, the quinone group, the moderately strong acid function, and the sugar, are all absent in the xanthoaphins; moreover, the spectrum [4,5] of the former bears no obvious relation to that of the latter or of the other aphins. Comparison of formulæ indicates that the change involves stoicheiometrically the hydrolysis of the glucosidic bond in protoaphin and a dehydration. It is in fact more complex still, as will be seen. The reaction proceeds under the extremely mild conditions of neutral pH and room temperature in the presence of the enzyme extract and cannot be brought about, other than in very poor yield, in any other way. Thus, although D-glucose can be isolated from hydrolysis of protoaphin with dilute acid,[6] the remainder of the molecule is converted largely into an amorphous powder which bears no resemblance to any of the fluorescent aphins. This may mean that once the sugar has been removed, the remaining stages in the conversion into xanthoaphin are also enzyme-controlled. The enzyme extract commonly used is not fractionated in any way, and enzymic functions additional to the glucosidase could well be present. Indeed the presence of a catalase in the extract can be shown easily by the quantitative liberation of oxygen on addition of hydrogen peroxide. Alternatively, failure to obtain fluorescent aphins by the action of acid on protoaphin may be due to lability of part of the protoaphin system under these conditions, preventing the rearrangement from occurring. A closer investigation of this and other aspects of the process is planned.

[11] Brockmann and Budde, *Chem. Ber.*, 1953, **86**, 432.

The entire protoaphin ⟶ xanthoaphin conversion can be envisaged in principle as taking place in three general stages (not necessarily in the sequence indicated): (*a*) hydrolysis of the glucosidic bond, (*b*) condensation of the naphthaquinone carbonyl with the now activated " resorcinol " ring; (*c*) formation of the hemiketal links, an analogy for which exists in the formation of pseudoskyrin derivatives [12,13] (IX) from skyrin. These possible stages are represented *schematically* (for the *fb* series) in the annexed chart.

There is very little doubt that step (*a*) occurs first and is enzymically controlled. Indeed we envisage the removal of glucose in this way as " triggering-off " the entire process by unmasking an additional hydroxyl group and so liberating the reactive naphthalene-1,3,8-triol system for further reaction. We do not know the specific sequence of events after step (*a*), nor do we know whether such events are brought about enzymically or not. A non-enzymic conversion of the protoaphin aglucone into xanthoaphin could be assisted by increased reactivity on removal of the sugar, and, since the two naphthalenic units in protoaphins cannot attain coplanarity for steric reasons, steric acceleration may also be a factor. The overall conversion is non-quantitative, a major side-reaction being probably the aerobic oxidation of the naphthalene-1,3,8-triol system in the aglucone. This is manifested as a non-stoicheiometric uptake (0·25 mole) of oxygen which accompanies the fermentation, and significantly higher yields (70%) of xanthoaphin can be obtained from protoaphin under nitrogen than in the presence of air (60%). This oxidation can be illustrated by investigating the glucosidic naphthalene fragment (glucoside B) obtained by reduction of the protoaphins.[2] In this case enzyme treatment

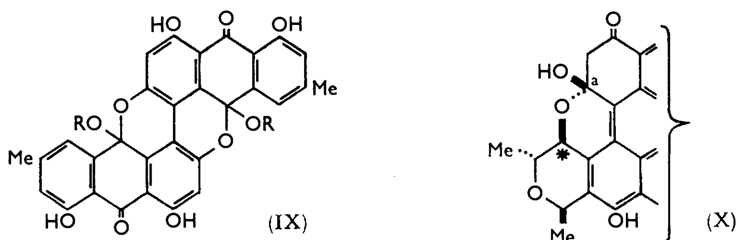


liberates the free naphthalene-1,3,8-triol, which cannot undergo processes analogous to xanthoaphin formation. The result is aerial oxidation at a rate comparable with that observed during protoaphin fermentation. It is complete within a few hours and corresponds ultimately to a total uptake of 1 mol. of oxygen.

The fact that the two naphthalene units in protoaphin cannot attain coplanarity introduces into the molecule an additional element of asymmetry, whose configurational aspects can now be discussed. Let us consider the stereochemistry of the protoaphin (I) ⟶ xanthoaphin (II) conversion, in conjunction with the structural observations made above. We have already noted that the only overall configurational change during this process is the production of two new centres of asymmetry, *viz.*, the carbon atoms (a, b) which in xanthoaphin (II) form part of the hemiketal groupings. Two stereochemical factors are involved in the formation of these new centres. First, as has already been observed, their configurations are determined by those of the neighbouring carbon atoms (marked *); an alternative configuration (X) would be impossible since C*, C^a, and C^b must all be in the plane of the anthracene system. Secondly, we believe that the attainment of this stable configuration can result only if the relative orientation of the two naphthalene nuclei in protoaphin (I) about their connecting bond, *i.e.*, the configuration due to restricted rotation, is such as to permit it. Two such configurations due

[12] Tanaka, *Chem. Pharm. Bull.* (*Japan*), 1958, **6**, 203.
[13] Shibata, Tanaka, and Kitagawa, *Pharm. Bull.* (*Japan*), 1955, **3**, 278.

to restricted rotation are possible. These can both most conveniently be represented by the protoaphin formula (I), in which the naphthalene glucoside is considered to be in the plane of the paper with the naphthaquinone making an angle with that plane. The non-aromatic side-chains attached to the naphthaquinone can then be directed either out of the plane, *i.e.*, towards the observer (isomer A), or into the plane, *i.e.*, away from the observer (isomer B). For xanthoaphin formation to occur in the case of A, the naphthaquinone would have to rotate relative to the glucoside, about the connecting bond in the sense shown in (I); in the case of B, rotation would be in the opposite sense. During this rotation serious interaction would occur between those alcoholic and phenolic hydroxyl groups in protoaphin, which together furnish the hemiketal system of xanthoaphin (we assume that hemiketal formation involves essentially attack by the two alcoholic hydroxyl groups on the neighbouring carbon atoms which bear the phenolic groups). The hemiketal at C^a is nearer to the axis of rotation than that at C^b and therefore interaction leading to its formation is likely to occur earlier. For both xanthoaphin-*fb* (II) and -*sl* (V), isomer A leads directly to the correct configuration at C^a; isomer B can yield only the unstable system (X). (The possibility that the latter system epimerises at some intermediate stage to the more stable one cannot be excluded, though we regard it as unlikely.) We therefore favour isomer A as representing both protoaphins-*fb* and -*sl*. Hemiketal formation at C^b is less amenable to arguments of this kind. This may well be because interactions leading to its formation cannot occur, for steric reasons, until the naphthaquinone system has been destroyed. If this is so, non-aromatic intermediates of as yet unknown structure must be involved and, consequently, the protoaphin molecule is an inadequate model on which to consider the process.



With the exception of some relatively minor points, *e.g.*, the configuration of the glucosidic link in the protoaphins and the detailed stereochemistry of chrysoaphins-*sl*-1 and -2, the elucidation of the structure and absolute stereochemistry of the aphins is now complete. Several associated problems, however, remain to be solved and work on them will be reported in due course.

Experimental

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

Paper Chromatography.—Methods were as previously described,[7] with mixtures of chloroform, light petroleum, and water: solvent A, 4 : 1 : 1; solvent B, 1 : 1 : 1.

Xanthoaphin fb.—(*a*) Protoaphin-*fb* was treated with pigment-free enzyme solution [4] as in (*b*) and (*d*) below. Extraction with ether yielded *xanthoaphin*-fb which was recrystallised from pure dry ether, dried at room temperature *in vacuo* for 6 hr., and analysed immediately (Found: C, 66·2; H, 5·2. $C_{30}H_{26}O_{10}$ requires C, 65·9; H, 4·8%); it had $\lambda_{max.}$ 258, 283, 359, 379, 406, 430, and 459 mμ (log ε 4·66, 4·74, 4·03, 4·33, 3·78, 4·04, and 4·08), the infrared absorption has been given previously,[8] and R_F in solvent A, 0·12, and B, 0·02.

(*b*) Protoaphin-*fb* (1·1 mg.) in phosphate–citrate buffer (pH 6·3; 4 ml.) and ethanol (1 ml.)

was treated with a few drops of pigment-free enzyme,[4] and the spectral range 350—750 mμ was scanned initially at 4-minute intervals for 2 hr. No intermediate between proto- and xanthoaphin could be detected.

(*c*) Protoaphin-*fb* (41·7 mg.) was placed in the side-arm of a microhydrogenator containing air. When equilibration was complete it was added to a mixture of enzyme solution [4] (20 ml.) and buffer (pH 6·3; 15 ml.). Oxygen uptake proceeded smoothly to a total volume of 0·22 ml. (*i.e.*, 0·25 mol. per mol. of protoaphin) in 220 min.

(*d*) Weighed samples of protoaphin (5 mg.) were treated in sealed tubes with enzyme solution [4] (2 ml.) and buffer (pH 6·3; 3 ml.) for 2·5 hr. at room temperature with occasional shaking. The mixtures were then extracted with ether (5 × 18 ml.), the extracts were made up to 100 ml., and xanthoaphin was estimated by optical density at 458 mμ. Experiments were performed both in an atmosphere of purified nitrogen and in air. In the former case the percentage conversions for a series of experiments were 73, 70, 69, 70, 66%, and in the latter 60, 67, 60, 52, 60%.

Xanthoaphin-sl.—(*a*) The ethereal mother-liquor from the crystallisation of chrysoaphin-*sl*-1 (below) was diluted with carbon tetrachloride and set aside at 0°; *xanthoaphin*-sl separated as bright yellow plates. Recrystallised from ether it had m. p. 206° (decomp.) and R_F 0·32 in solvent A and 0·07 in B (Found: C, 65·8; H, 4·8. $C_{30}H_{26}O_{10}$ requires C, 65·9; H, 4·8%). Light absorption was as for xanthoaphin-*fb*. Infrared absorption was 3280, 1626, 1590, 1545, 1344, 1316, 1305, 1282, 1242, 1202, 1190, 1170, 1145, 1115, 1104, 1083, 1064, 1033, 1015, 974, 961, 950, 923, 899, 833, 828, 780, 769, 760, 697, and 668 cm.$^{-1}$.

(*b*) Protoaphin-*sl* (3 mg.) was treated with enzyme [4] as for the *fb* isomer. Extraction with ether and paper chromatography showed the presence of only one xanthoaphin, R_F as above.

Chrysoaphin-fb.—A sample prepared as described in Part II [5] recrystallised from ether as fine orange plates, m. p. 250° (decomp.) (Found: C, 68·0; H, 5·2. Calc. for $C_{34}H_{24}O_9$: C, 68·2; H, 4·6%), $\lambda_{max.}$ 268, 380, 402, 457, and 485 mμ (log ε 4·64, 4·30, 4·51, 4·19, and 4·21), $\nu_{max.}$ 3330, 2967, 2915, 1642, 1613, 1590, 1575, 1481, 1416, 1366, 1292, 1282, 1258, 1244, 1206, 1183, 1163, 1155, 1131, 1093, 1070, 1064, 1031, 1000, 951, 896, 875, 866, 852, 833, 806, 787, 774, 761, 733, 719, 704, and 694 cm.$^{-1}$, R_F in solvent A, 0·65, B, 0·18.

Mixed Chrysoaphins-sl.—(*a*) A solution of xanthoaphin-*sl* (3 mg.) in ether (10 ml.) was treated with dilute ethanolic sulphuric acid (2 ml.) for 2 min., then extracted with aqueous sodium hydrogen carbonate, washed with water, and dried. The product had R_F in solvent B, 0·06 (xanthoaphin-*sl*), 0·32 (chrysoaphin-*sl*-2), and 0·44 (chrysoaphin-*sl*-1), the last two of comparable intensity.

(*b*) Experiment (*a*) was repeated with xanthoaphin-*sl* (1·12 mg.), chloroform (100 ml.), and sulphuric acid in ether (0·1%; 0·5 ml.), and the spectral change were followed for 8 hr. at 90-min. intervals. No intermediates were observed.

Chrysoaphin-sl-1.—Mixed aphins-*sl* (0·3 g.), extracted from *Tuberolachnus salignus* as described in Part III,[6] were extracted from a Soxhlet thimble into boiling ether (150 ml.). Cooling yielded *chrysoaphin*-sl-1 as orange plates, m. p. 238—239° (decomp.) (Found: C, 67·9; H, 5·0. $C_{30}H_{24}O_9$ requires C, 68·2; H, 4·6%). Light absorption was as for chrysoaphin-*fb*. Infrared absorption was at 3240, 1638, 1612, 1587, 1552, 1515, 1307, 1290, 1277, 1235, 1189, 1166, 1156, 1144, 1111, 1096, 1068, 1034, 1006, 976, 950, 901, 877, 871, 838, 826, 809, 799, 788, 764, 699, 678, and 667 cm.$^{-1}$. R_F in solvent A was 0·75. Titration in dimethylformamide solution with tetra-n-butylammonium hydroxide (0·2N in benzene–propan-2-ol) gave Equiv., 519, 520 ($C_{30}H_{24}O_9$ requires Equiv., 528).

Chrysoaphin-sl-2.—A solution (0·1% w/v) of concentrated sulphuric acid in ether (2·5 ml.) was added to xanthoaphin-*sl* (50 mg.) in chloroform (10 ml.), and the mixture left at room temperature for 1 hr., then washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated under reduced pressure. The orange product was recrystallised from carbon tetrachloride. Paper chromatography (solvent A) showed it to be a mixture of two substances of R_F 0·75 (chrysoaphin-*sl*-1) and 0·63. This product (40 mg.) was dissolved in dioxan (30 ml.) and added in 1 hr. to boiling aqueous sodium metaperiodate (40 mg. in 25 ml. of water). The cooled mixture was extracted with ether, and the extract was washed, dried, and evaporated, to give a red resin which was chromatographed in benzene on silica gel. Benzene eluted in succession a red (erythroaphin-*sl*) and an orange band. Evaporation of the eluate of the latter band gave an orange solid (14 mg.) which, recrystallised from carbon tetrachloride, gave *chrysoaphin*-sl-2 (5 mg.) as an orange microcrystalline powder, m. p. 212—215° (decomp.),

with R_F in solvent A 0·65 (Found: C, 68·5; H, 5·0. $C_{30}H_{24}O_9$ requires C, 68·2; H, 4·6%). Light absorption was as for chrysoaphin-*fb*. Infrared absorption was at 3300, 2850, 1641, 1615, and 1595 cm.$^{-1}$.

Conversions of Chrysoaphins into Erythroaphins.—(*a*) A solution of chrysoaphin-*fb* (0·985 mg.) in chloroform (100 ml.) was treated with concentrated hydrochloric acid (0·2 ml.), and spectral changes were followed at hourly intervals for 8 hr. No intermediate was observed in the resulting smooth conversion into erythroaphin.

(*b*) Similar treatment of mixed chrysoaphins-*sl* gave a similar result.

Treatment of Enzyme with Peroxide.—Hydrogen peroxide (100-vol.; 36·5 mg.) was treated with an excess of enzyme solution (20 ml.), and gas evolution measured. This was complete in 1·5 hr. and amounted to 3·74 ml., *i.e.*, 0·55 mole per mole of peroxide.

Tetrachloroxanthoaphin-fb.—(*a*) A rapid stream of chlorine was passed through a solution of erythroaphin-*fb* (27 mg.) in nitrobenzene (3 ml.) at 100° for 8 min. During this period the intense red colour of the solution faded to a pale orange-yellow; prolonged passage of chlorine caused darkening and decomposition. The excess of chlorine and the nitrobenzene were removed under reduced pressure at $<80°$, leaving an orange gum which crystallised on trituration with carbon tetrachloride. Recrystallised from carbon tetrachloride *tetrachloroxanthoaphin-fb* formed bright yellow plates (16 mg.), R_F in solvent A, 0·36. The crystalline material contained carbon tetrachloride which was tenaciously held and could not be completely removed at 140° without decomposition [Found, in material dried at 100°/10^{-3} mm.: C, 44·3; H, 2·6; Cl, 35·0 (Carius). $C_{30}H_{22}Cl_4O_{10},CCl_4$ requires C, 44·4; H, 2·6; Cl, 34·0%]. It had $\lambda_{max.}$ 265, 284, 363, 383, 408, 432, and 461 mμ (log ε 4·91, 4·43, 4·09, 4·34, 3·83, 4·09, and 4·14), and $\nu_{max.}$ 3290, 1657, 1615, 1550, 1302, 1284, 1237, 1192, 1164, 1134, 1112, 1070, 1058, 1030, 953, 895, 861, 832, 786, and 765 cm.$^{-1}$, the bands at 786, 765 cm.$^{-1}$ being characteristic of CCl_4. The substance dissolved readily in chloroform and benzene, to give yellow non-fluorescent solutions. When crystallised from benzene it retained solvent of crystallisation which could be detected spectroscopically. It gave a violet solution in concentrated sulphuric acid and was unaffected by hot formic acid.

(*b*) Similar chlorination of dibromoerythroaphin-*fb* (55 mg.) yielded tetrachloroxanthoaphin-*fb* (35 mg.), identical in spectra and in R_F with the product from (*a*) (Found: C, 43·9; H, 2·4; Cl, 34·6%).

(*c*) Similar chlorination of di-iodoerythroaphin-*fb* (50 mg.) yielded a similar yellow amorphous solid (30 mg.). The presence of tetrachloroxanthoaphin was detected spectroscopically; R_F in solvent A, 0·37.

Tetra-acetyltetrachloroxanthoaphin-fb.—A solution of tetrachloroxanthoaphin-*fb* (100 mg.) in acetic anhydride (3·7 ml.) and pyridine (2·3 ml.) was left overnight at room temperature. The resulting brown mixture was diluted with benzene (120 ml.), washed successively with water, 2N-hydrochloric acid, and water, dried, and evaporated and the yellow residue (53 mg.) recrystallised twice from chloroform–ethanol. The *acetyl derivative* formed yellow prisms (37 mg.), m. p. 250° (decomp.) (Found, in material dried at 75°/10^{-3} mm.: C, 53·8; H, 4·0; Cl, 17·4; *O*-acetyl, 19·2. $C_{38}H_{30}Cl_4O_{14}$ requires C, 53·6; H, 3·5; Cl, 16·7; *O*-acetyl, 20·2%.) Light absorption max. were at 255, 279, 365, 385, and 424 mμ (log ε 4·44, 4·84, 3·77, 4·10, and 3·96). Infrared absorption was at 1779 (aromatic Ac), 1750 (aliphatic Ac), 1717, 1615, 1535, 1342, 1314, 1297, 1275, 1244, 1223, 1206, 1177, 1155, 1096, 1080, 1035, 1003, 986, 940, 914, 880, 854, 808, 803, and 743 cm.$^{-1}$.

Reduction of Tetrachloroxanthoaphin-fb.—(*a*) A solution of tetrachloroxanthoaphin-*fb* (21 mg.) and fused sodium acetate (50 mg.) in dioxan (10 ml.) was hydrogenated over platinum oxide (50 mg.). When the hydrogen was replaced by air the solution quickly changed its colour from yellow to deep orange-red; it was filtered, diluted with water (100 ml.) and N-hydrochloric acid (25 ml.), and extracted with ether. The extract ($\lambda_{max.}$ 249, 404, 455, and 485 mμ, similar to a chrysoaphin) [5] was washed, dried, and evaporated and the red residue was refluxed for 3 hr. with ethyl acetate (30 ml.) and formic acid (5 ml.). Solvents were removed under reduced pressure and the dark red gummy residue was dissolved in chloroform (200 ml.). The pigment was extracted from the chloroform by shaking it with 75% w/w sulphuric acid (2 × 50 ml.); after dilution of the acid solution with 50% w/w sulphuric acid (100 ml.), the pigment was extracted from it with chloroform (4 × 75 ml.). The chloroform solution was washed, dried, concentrated to small bulk, and diluted with ethanol. Dichloroerythroaphin-*fb* [9] separated gradually and was recrystallised from chloroform–ethanol (Found, in

material dried at 75°/10^{-3} mm.: C, 56·6; H, 3·3. Calc. for $C_{30}H_{20}Cl_2O_8,0{\cdot}5CHCl_3$: C, 57·4; H, 3·2%). The product was identical in spectra with authentic material.

(*b*) Tetrachloroxanthoaphin-*fb* (12 mg.) was reduced with zinc and acetic acid as described previously.[9] The product recrystallised from chloroform–ethanol, to give dichloroerythroaphin-*fb* (4 mg.), identified by spectral comparison.

*Reductive Acetylation of Tetra-acetyltetrachloroxanthoaphin-*fb.—A solution of tetra-acetyltetrachloroxanthoaphin-*fb* (40 mg.) and fused sodium acetate (25 mg.) in acetic anhydride (7 ml.) was hydrogenated over Adams catalyst (40 mg.). The solution was filtered and evaporated under reduced pressure. The resulting gum was chromatographed in benzene on silica. Elution with benzene–ethyl acetate (20 : 1) gave a yellow eluate fraction with an intense green fluorescence. Evaporation gave an orange gum which after being crystallised twice from chloroform–ethanol gave tetra-acetyldihydrodichloroerythroaphin-*fb* (10 mg.) as yellow needles, whose identity was confirmed spectroscopically. Hydrolysis of a sample (4 mg.) with methanolic sodium hydroxide as described previously yielded dichloroerythroaphin-*fb* (2 mg.), identified by its ultraviolet and infrared spectra.[9]

*Tetrachlorotetrahydroxanthoaphin-*fb.—Sodium borohydride (207 mg.) was added to a solution of tetrachloroxanthoaphin-*fb* (90 mg.) in methanol (80 ml.), and the mixture was set aside at room temperature overnight. The resulting orange solution, which had an intense green fluorescence, was poured into an excess of cold N-hydrochloric acid. The mixture was extracted with ether, and the extract, which was yellow with a violet fluorescence, was washed, dried, concentrated, and diluted with light petroleum (b. p. 40—60°). *Tetrachlorotetrahydroxanthoaphin-*fb (45 mg.) separated as a yellow powder which was twice reprecipitated from ether with light petroleum before analysis (Found: C, 51·7; H, 4·2. $C_{30}H_{26}Cl_4O_{10}$ requires C, 52·4; H, 3·7%. It had R_F in solvent A 0·36, $\lambda_{max.}$ 271, 278, 344, 363, 380, 405, and 427 mμ (log ε 4·72, 4·82, 3·84, 3·91, 3·95, 3·94, and 3·91), and $\nu_{max.}$ 3350, 1634, 1565, 1345, 1310, 1260, 1156, 1136, 1113, 1073, 1044, 1021, 958, 927, 903, 867, 822, and 808 cm.$^{-1}$.

This compound (35 mg.) was left for 24 hr. at room temperature in pyridine (3 ml.) and acetic anhydride (3 ml.). Solvents were removed under reduced pressure and the residue was chromatographed in benzene on silica gel. Elution with benzene containing 7% of ethyl acetate gave a yellow, green-fluorescent solution. Evaporation followed by recrystallisation from ether–light petroleum (b. p. 40—60°) gave the yellow microcrystalline *hexa-acetate* (7 mg.) (Found: C, 54·0; H, 4·2. $C_{42}H_{38}Cl_4O_{16}$ requires C, 53·7; H, 4·1%), $\lambda_{max.}$ 269, 278, 319, 376, 393, and 416 mμ (log ε 4·66, 5·01, 3·71, 3·95, 4·07, and 3·98), $\lambda_{infl.}$ 360 mμ (log ε 3·71), and $\nu_{max.}$ 1760, 1630, 1307, 1287, 1220, 1166, 1135, 1115, 1080, 1030, 1005, 950, 925, 867, 840, 800, and 783 cm.$^{-1}$.

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