Colouring Matters of the Aphididae. Part XIV. The Course of Substitution Reactions and the Stereochemistry of the Erythroaphins.*

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The course of the interaction of erythroaphin with amines, alkaline potassium permanganate, and bromine is discussed and a stereochemical explanation offered for the conversion of erythroaphin-*sl* into derivatives of the *fb*-series observed with the first two of the above reagents but not with bromine. The arguments are extended to include the results of the Thiele acetylation of erythroaphin-*sl* where inversion does not occur and of monohydroxyerythroaphin-*sl* where it does. It is postulated that the *fb*-series have both pairs of reduced rings fused in the *cis*-configuration and that in the *sl*-series, one pair is *cis* and the other *trans*. Ultra-violet irradiation of the tetra-acetyldihydroerythroaphins-*fb* or -*sl*, as well as the penta-acetyldihydrohydroxyerythroaphins-*fb* or -*sl*, causes racemisation at the optically active centres and from the irradiation of *cis-cis*-tetra-acetyldihydroerythroaphin (*fb*-series), the *cis-trans*-isomer (*sl*-series) has been isolated as well as the hitherto unknown *trans-trans*-isomer. A consideration of the relative configuration at the four asymmetric centres leads to a complete structure for each of the isomers.

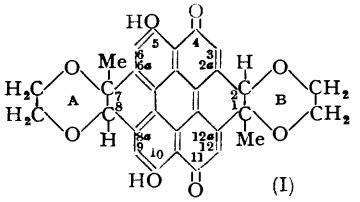
In an earlier paper (Part XIII) the alternative structures (I) and (II) were advanced for erythroaphin, the red pigment of the aphin series. Two well-defined series of aphins have been isolated from natural sources: the *fb*-series from *Aphis fabae* and several other species, and the *sl*-series from *Tuberolachnus salignus*. Strong evidence has been provided (Part XII, *J.*, 1955, 954) for the view that the two erythroaphins are stereoisomers and the relation between erythroaphin-*fb* and erythroaphin-*sl* can now be examined in the light of the basic erythroaphin structure (I).† This structure will be adopted in preference to (II) on the grounds that a plausible explanation for the course of the substitution reactions and interconversions of the isomers can be developed only on the basis of (I).

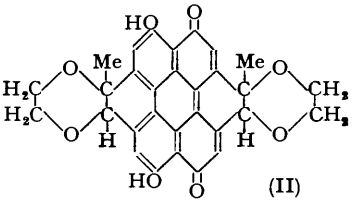
Both erythroaphins are optically active. Owing to the intense colour of their solutions, accurate measurement of their optical rotation is difficult, but by using an intense white source erythroaphin-*fb* was found to have a specific rotation of approximately +20°

* Part XIII, *J.*, 1955, 959.

† Numbering of the complete ring system is postponed. The numerals used in the present discussion refer only to the coronene numbering shown in formula (I). Complete enumeration of the molecule awaits final clarification of the structure.

and erythroaphin-*sl* of +150° (±15°) (both in $CHCl_3$). The situation is much the same with the two diacetylerythroaphins which, in acetone, have specific rotations of approx. 0° (*fb*), and +95° (±15°) (*sl*), and the two dibromoerythroaphins which have [α] +25° (*fb*) and +150° (*sl*) (in $CHCl_3$), but the two tetra-acetyldihydro-derivatives, which give


(I)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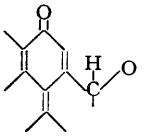
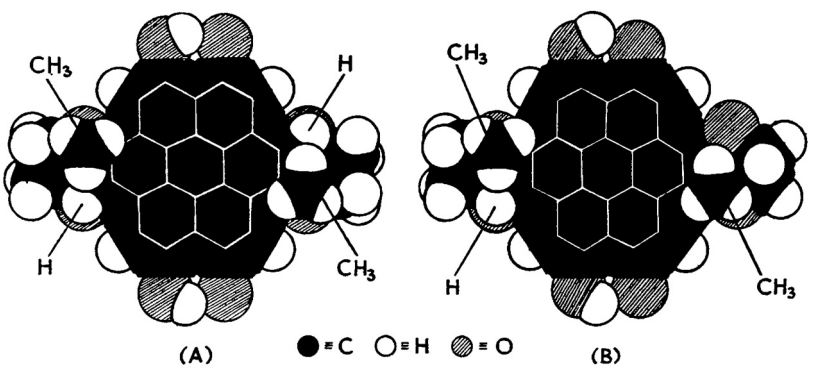

(III)

yellow solutions, are more easily studied. When freshly prepared, tetra-acetyldihydro-erythroaphin-*fb* has $[\alpha]_D$ −560° and the *sl*-isomer has $[\alpha]_D$ +95° (both in C_6H_6). Provided that light is excluded, these rotation values are independent of the method of preparation of the tetra-acetyldihydro-derivative, *e.g.*, reductive acetylation of either erythroaphin or diacetylerythroaphin with zinc and acetic anhydride or catalytic reduction of erythroaphin either in the presence of acetic anhydride or with subsequent acetylation.

In structure (I) carbon atoms 1, 2, 7, and 8 are asymmetric. The conversion of erythroaphin-*sl* into diamino- or dihydroxy-erythroaphin-*fb* (Part XII, *loc. cit.*) must therefore involve an optical inversion at one or more of these four centres. It is noteworthy that this inversion occurs as a result of chemical reactions involving reduction and re-oxidation and also the introduction of groups larger than hydrogen; the only mechanism of stereochemical change which seems reasonable is that involving a hydrogen atom or atoms attached to one or more of the four asymmetric centres, these being located in relation to the quinone carbonyl groups as in the partial structure (III). The racemisation of centres bearing hydrogen in such positions is already known in simpler quinone molecules (cf., *e.g.*, Schmidt and Ebnöther, *Helv. Chim. Acta*, 1951, **34**, 561). In the case of erythroaphin-*sl*, however, it is optical inversion and not merely racemisation which occurs, and since it always accompanies the entry of two amino- or hydroxy-groups into the molecule, the inversion would appear to be brought about by some steric consideration

FIG. 1. *Erythroaphin :* (*A*) cis-cis-*fusion of dioxan rings;* (*B*) cis-trans-*fusion of dioxan rings.*



enforced by their presence. The existence of the erythroaphins-*sl* and -*fb* as separate stable entities even under acid conditions (under alkaline conditions addition reactions occur with great rapidity) is not surprising. Although (I) is written formally as a dihydroxy-quinone, it is so strongly hydrogen bonded that it does not contain either a simple quinonoid or a dihydroxylic system (cf. infra-red spectroscopic evidence; Johnson, Quayle, Robinson, Sheppard, and Todd, Part V, *J.*, 1951, 2633) so that inversion or racemisation of centres 1, 2, 7, or 8 is unlikely to occur so easily in the erythroaphins themselves by the mechanism under discussion. In this connection it is of interest that, whereas dipiperidinoerythroaphin-*fb* is stable towards strong acids (Part VII, Brown,

Johnson, MacDonald, Quayle, and Todd, *J.*, 1952, 4928), the diacetyl derivative, in which the quinone carbonyl groups are no longer hydrogen-bonded, is hydrolysed by dilute hydrochloric acid to yield the diacetyl derivative of dihydroxyerythroaphin-*fb*.

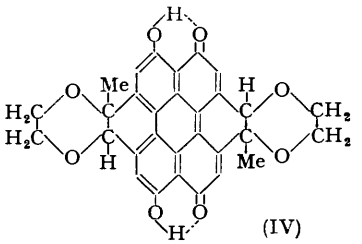
Examination of molecular models of structure (I) (Fig. 1) shows that there are two possible ways of attaching each dioxan ring to the rest of the molecule. In one form the methyl group and the hydrogen atom on the junction carbons are in a *cis*-relation to each other and one of the oxygen atoms in the dioxan ring (chair form) lies well above the plane of the perylene system. In the other form both oxygens lie much more nearly in the plane of the aromatic system and the hydrogen atom and methyl group are in a *trans*-relation. For convenience we will refer to these two dioxan ring arrangements as *cis*- and *trans*-rings. A point of particular interest is that if, in (I), ring B is *cis* with the oxygen adjacent to the CH-group above the plane of the aromatic portion, then position 3 is virtually unhindered and groups such as amino may be introduced freely, whereas there is appreciable hindrance to their introduction at 12. With ring B in the *trans*-form, however, the oxygen atoms of the dioxan ring interfere appreciably with the entry of amino-groups either at position 3 or at position 12. It is to this fact that we attribute the optical inversion which causes the change from the *sl*- to the *fb*-series in the amination and hydroxylation reactions and we formulate the reactions involved in the following way.

In erythroaphin-*fb* (IV) we consider that both A and B are "*cis*-rings," whereas, in erythroaphin-*sl* (V), ring A is a "*trans*-ring" and B a "*cis*-ring." It is clear that formula (IV) could represent either of two diastereoisomers—a *cis-syn-cis*-compound in which both angular methyl groups lie on the same side of the central perylene system and a *cis-anti-cis*-isomer in which they are opposed. Equally there could be a *cis-syn-trans*- and a *cis-anti-trans*-isomer of (V). The stereochemistry of the erythroaphins will be discussed in full later in this paper but interpretation of the interconversion reactions requires only an assumption that the disposition of the angular methyl groups relative to one another (*i.e.*, *syn* or *anti*) be the same in erythroaphin-*fb* (IV) as in erythroaphin-*sl* (V). On treatment with ammonia, reaction proceeds by the usual quinone mechanism of addition followed by reoxidation and entry of a second group by the same process. The first addition will clearly take place in such a way that the amino-group takes up a sterically unhindered position, *i.e.*, it will enter at position 3 in each case (in erythroaphin-*fb* $C_{(3)}$ is equivalent to $C_{(9)}$). On subsequent re-oxidation we assume that in accordance with the known effect of amino-groups (*e.g.*, Evans and de Heer, *Quart. Rev.*, 1950, **4**, 94) the product will behave in its further reaction like a true quinone with one carbonyl in the same ring as the amino-group, *i.e.*, at $C_{(4)}$. The second carbonyl group might be located at $C_{(10)}$ or $C_{(11)}$. There are no suitable analogies in the literature on polycyclic hydroxyquinones which bear on this point but it would seem at any rate probable that the next point of attack in the molecule would be $C_{(9)}$ or $C_{(12)}$, $C_{(9)}$ being preferred on steric grounds and the 4 : 10-quinone being assumed to this end. This being so, entry of the second amino-group would be at position 9. This position is favourable in the case of the 3-amino-derivative of the *fb*-series, but it is sterically hindered in the *sl*-intermediate (VI), being adjacent to a *trans*-dioxan ring. Since, however, the hydrogen atom attached to $C_{(8)}$ in (VI) is suitably activated by the 10-quinone carbonyl group, it is suggested that during the removal of the proton from $C_{(8)}$ and amination at $C_{(9)}$ inversion of configuration occurs, so converting ring A into a *cis*-ring, *i.e.*, it yields diamino-erythroaphin-*fb* (VII) at the second amination. It is clear that a similar explanation of the inversion and amination processes could be given if the intermediate 3-amino-quinone contained the 5 : 10-quinone system but there is as yet no direct evidence to bring to bear on this point. Likewise, whether the actual inversion process occurs during or subsequent to the entry of the second group cannot be established with certainty. Similar considerations will apply to the formation of substituted diamino-derivatives and dihydroxy-derivatives, conversion of the *sl*- into *fb*-series being uniformly observed.

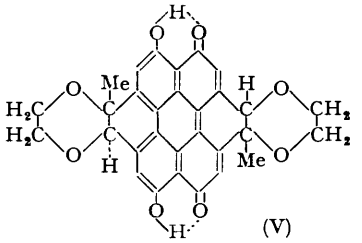
Erythroaphin-*fb* and erythroaphin-*sl* on bromination give dibromo-derivatives which are not identical, but amination of either of these derivatives yields diamino-dibromo-erythroaphin-*fb*, interconversion having occurred in the *sl*-series (Part XII, *J.*, 1955, *loc.*

cit.). These facts can also be explained on the basis of formulæ (IV) and (V). In the bromination of erythroaphin, unlike the amination, ionisation at position 2 or 8 does not occur and consequently the configuration at these asymmetric centres is retained. We may reasonably assume that the first bromine to enter the molecule will in each case do so at the unhindered position 3. Although no definite evidence is available, it seems most likely that the further action of bromine would cause substitution at position 9, giving a symmetrical structure; position 12 is a possible alternative but position 6 is most unlikely. If the dibromo-derivatives are assumed to be 3 : 9-dibromoerythroaphins, then subsequent reaction with amines would introduce amino-groups at positions 6 and 12. That an inversion should occur during this process in the case of the *sl*-isomer is not surprising, since, movement of the hydrogen atoms at the dioxan ring junctions being possible, the tendency will be for production of the configuration (*i.e.*, that of *fb*) in which steric hindrance is least.

In the absence of knowledge as to the precise position of substituents in the substituted erythroaphins it is impossible to do more than advance hypothetical explanations of their interconversion. Although we have formulated both the diamino- and the dibromo-erythroaphins with the substituents in the 3 : 9-positions as though derived from intermediate 3-substituted perylene-4 : 10-quinones, spectroscopic evidence favours a 4 : 11-(or 5 : 10)-quinone structure for the acetylated disubstituted products. Despite all efforts, we have been unable to dehalogenate diaminodibromoerythroaphin-*fb* without affecting the amino-groups and so are unable to say with any certainty whether the amino-groups in that compound occupy the same positions as in diaminoerythroaphin-*fb*, or indeed whether in the latter they occupy the 3 : 9- or 3 : 12-positions. Nevertheless we consider that, on the basis of formulæ (IV) and (V) for erythroaphin-*fb* and erythroaphin-*sl*, and assuming that diaminoerythroaphin-*fb* is (VII), a reasonable explanation of the observed facts can be given, and that, as above indicated, the properties of the dibromoerythroaphins may also be explained.



(IV)



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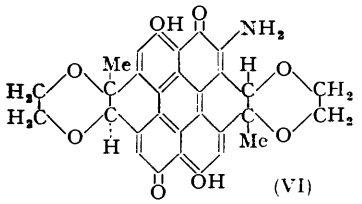
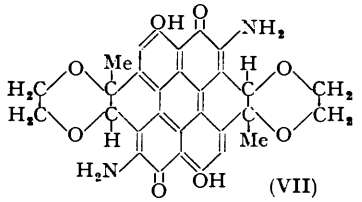


(VI)



(VII)

It may be pointed out that the stereochemical relation of the erythroaphins-*fb* and -*sl*, *i.e.*, *cis-cis* and *cis-trans* respectively, has been deduced independently on the bases of the quantitative acetaldehyde production (Part XIII, preceding paper) and also in the present paper from conditions of the addition reactions which bring about the inversion of erythroaphin-*sl*.

On our hypothesis outlined above, the two erythroaphins should yield distinct mono-amino- and monohydroxy-derivatives since optical inversion should occur only upon the entry of a second substituent. Unfortunately we have never been able to halt reaction with ammonia or amines at the monosubstituted stage, but monohydroxy-derivatives are accessible by means of the Thiele acetylation procedure followed by hydrolysis and aerial oxidation of the initially formed penta-acetyldihydrohydroxyerythroaphins. In the

original work, it was observed that hydroxyerythroaphin-*fb* and -*sl* were extremely alike but always showed a slight difference in their infra-red absorption spectra (Part XII, *loc. cit.*). Although on this basis they were adjudged to be distinct substances, the puzzling observation was made that treatment with zinc dust and acetic acid converted both of them in rather poor yield into a product identified by infra-red spectrum as erythroaphin-*fb*, although reduction alone had never previously been known to convert a member of the *sl*-series into an *fb*-derivative. Subsequent work on the penta-acetyldihydrohydroxyerythroaphins has helped to remove this apparent anomaly. It was found that the optical rotations of the freshly prepared *fb*- and *sl*-derivatives differed considerably ($[\alpha]_D$ −230° and −40° respectively) but that solutions exhibited a very ready mutarotation in the presence of light. The change in rotation was greatly accelerated when the solutions were irradiated and both penta-acetyldihydro-compounds yield, at equilibrium (after *ca.* 24 hours), solutions of substantially the same rotation (−30°). The penta-acetyl derivatives are unfortunately rather unstable and no pure compounds have been isolated from the solution after equilibration by irradiation. Nevertheless it seems clear that the effect of light is to cause racemisation at one or more of the asymmetric centres and thus an explanation is provided for the anomaly presented by the hydroxyerythroaphins. The process of preparing them lasts several days and no

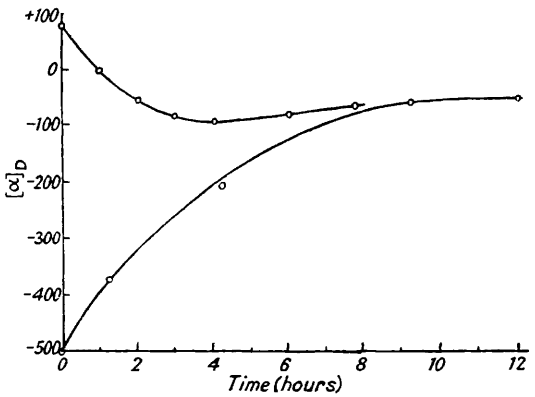


FIG. 2. *Effect of ultra-violet irradiation on the optical rotation of benzene solutions of the tetra-acetyldihydroerythroaphins*-fb *and* -sl.

effort was made in the original experiments to exclude light. As a result the hydroxy-erythroaphins isolated would be expected to be mixtures containing perhaps a majority of that derivative corresponding to the original aphin. Hence the difference between the products in the *fb*- and the *sl*-series would be unexpectedly small, and treatment with zinc and acetic acid, followed by normal processes of recrystallisation, would give, as the final product from each, the least soluble aphin, *viz.*, erythroaphin-*fb*. A repetition of the Thiele acetylation process with the hydroxyerythroaphin-*fb* and -*sl* gave apparently identical products, *i.e.*, hexa-acetyldihydrodihydroxyerythroaphin, as judged by the optical rotations, but extensive decomposition occurred during the reaction and the product was not isolated in a pure state. However, this experiment gave further support to the theory of the nature of the conversion of the *sl*- into the *fb*-series.

In following up observations that samples of tetra-acetyldihydroerythroaphin-*sl* sometimes varied considerably in their optical rotation, it was discovered that solutions both of this substance and of the corresponding *fb*-derivative also exhibit mutarotation in the presence of light. The change in rotation was markedly slower than in the case of the penta-acetyldihydrohydroxyerythroaphins and the compounds were themselves more stable, so that the tetra-acetyldihydroerythroaphin series was more convenient for a study of the nature of the mutarotation. As before, the change was slow in diffused daylight but much more rapid when the solutions were irradiated with ultra-violet light, both isomers yielding, at equilibrium, solutions with substantially the same optical rotation (*ca.* −50°; Fig. 2), although the *sl*-isomer attained the equilibrium value appreciably more rapidly than the *fb*-isomer. It is of interest to consider these observations in the light of the proposed structures (IV) and (V) for erythroaphin-*fb* and -*sl*. In the

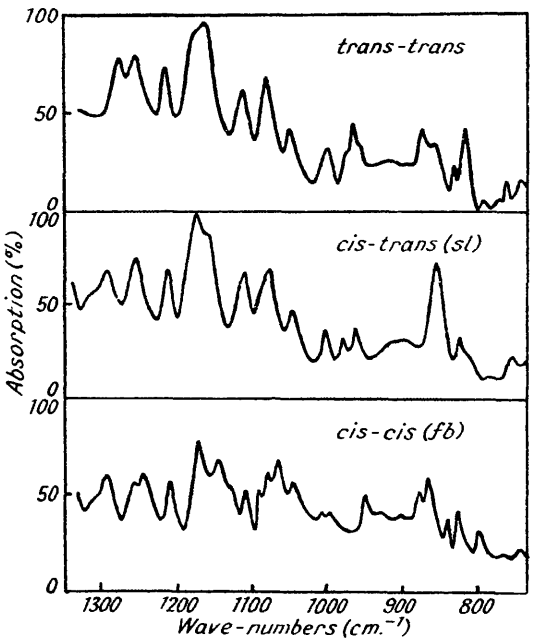
unsubstituted erythroaphins, or in the tetra-acetyldihydro-derivatives, there is no steric factor to impose inversion rather than racemisation at the asymmetric centres. We would therefore assume that the effect of ultra-violet irradiation would be to cause racemisation at positions 2 and 8 in each case. This, because of the symmetry of the molecule, would lead to the production of three diastereoisomers corresponding to erythroaphins-*fb*, -*sl*, and a third erythroaphin in which both A and B are "*trans*-rings." Tetra-acetyldihydroerythroaphin-*fb* (the least soluble component), as well as unchanged *sl*-isomer, has been isolated from the equilibrium mixture prepared by irradiation of tetra-acetyldihydroerythroaphin-*sl* and when, in a larger-scale experiment, tetra-acetyldihydroerythroaphin-*fb* (*cis-cis*) was irradiated with ultra-violet light, there was isolated from the more soluble fractions not only the *sl*(*cis-trans*)-isomer but also the hitherto unknown *trans-trans*-isomer. Hydrolysis of the *trans-trans*-tetra-acetyldihydro-compound ($[\alpha]_D$ +380°) gave the corresponding *trans-trans*-erythroaphin which, as yet,

TABLE.

Bands in the 1000—800 *cm.*$^{-1}$ *region in the infra-red spectra of the three isomers of erythroaphin.*

cis-cis (*fb*)	*cis-trans* (*sl*)	*trans-trans*
952	974	964
878	955	870
864	852	829 (weak)
842	818	813
830		
805		

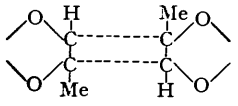
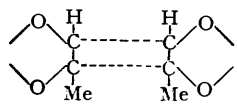
FIG. 3. *Infra-red spectra of the erythroaphin isomers.*



has not been isolated from any species of aphid. It is more soluble in organic solvents than the others and when examined in a white light has a high positive rotation ([α] +325°). There is thus a gradation of physical properties, especially solubilities and optical rotation, from the least soluble tetra-acetyldihydroerythroaphin-*fb* with a negative rotation through the *sl*-isomer to the most soluble *trans-trans*-isomer with a high positive rotation. Apart from differences in optical rotation, the three erythroaphins can be distinguished by their infra-red spectra (Fig. 3), particularly in the 1000—800 cm.$^{-1}$ region (see Table above).

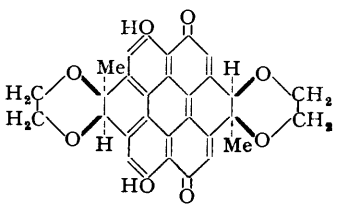
As all three of the isomers of erythroaphin are optically active, a reconsideration of structures (I) and (II) makes it possible to eliminate certain of the stereochemical isomers on the grounds of symmetry. In the aromatic portion of the molecule either of the hydrogen atoms of the two hydroxyl groups is almost symmetrically placed with regard to the carbonyl and hydroxyl oxygen atoms. The energy involved in the transfer of hydrogen from one oxygen to the other is relatively small and a state of dynamic equilibrium is assumed in the *peri*-hydroxy-quinone system. Consequently this system will not of itself confer asymmetry on the molecule and for the purpose of stereochemical considerations the perylene system can be ignored, leaving the groups (VIII) or (IX),

depending on whether the angular methyl groups are situated at positions 1 and 7 or at positions 1 and 8. The situation thus obtained recalls that of the truxinic and truxillic acids except that the four asymmetric carbon atoms are arranged as a rectangle rather

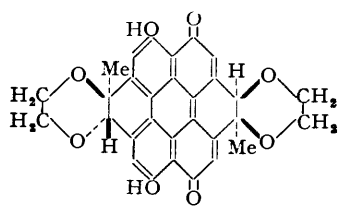
(VIII)   (IX)

than a square. In (VIII), an arrangement with the methyl groups in the *trans*-position relative to each other (*anti*-forms) can be eliminated because both the *cis-cis*- and *trans-trans*-erythroaphin isomers would then have a centre of symmetry. Hence on the basis of (VIII), the structures (X) and (XI), *i.e.*, with the methyl groups in the *cis*-position relative to each other (*syn*-forms), must represent erythroaphin-*fb* and -*sl* respectively.

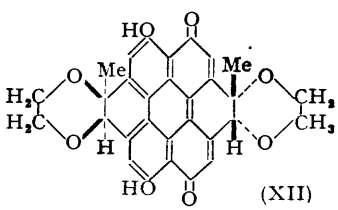
From what has been said previously, structure (I) is preferred to (II) for erythroaphin but it should be noted that if the angular methyl groups are arranged as in (II) (or IX) they must be in the *trans*-position relative to each other; otherwise *cis-cis*- and *trans-trans*-erythroaphin would each have a plane of symmetry. On this basis structures (XII) and (XIII) would be assigned to the erythroaphins-*fb* and -*sl* respectively.



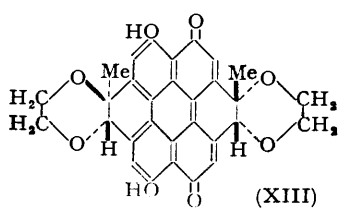
(X) (*cis-syn-cis*)



(XI) (*anti-syn-cis*)



(XII)



(XIII)

Experiments designed to show the relative positions of the methyl groups in the erythroaphin molecule and thus remove all ambiguity as to the complete stereochemical structure of all three isomers, are at present in progress.

Experimental

Dibenzoylerythroaphin-sl.—To erythroaphin-*sl* (55 mg.), dissolved in dry pyridine (6 c.c.), at <10° benzoyl chloride (1·0 c.c.) was added dropwise with shaking. The mixture was allowed to warm to room temperature and kept for a further 15 min. with occasional shaking; then the orange solution was diluted with ether (30 c.c.) and poured on ice. The ethereal layer was separated, washed, dried, and concentrated (to 5 c.c.), and then heated on the steam-bath for 5 min. with ethanol (20 c.c.) in order to convert the residual benzoyl chloride into ethyl benzoate. After cooling, the product separated as orange crystals which were collected, washed with ethanol, and dried (60 mg.). It was recrystallised from a mixture of benzene (3 c.c.) and light petroleum (b. p. 40—60°; 15 c.c.) and further purified by chromatography in benzene (3 c.c.) on silica (7 × 2 cm.). The column was eluted with benzene–acetone (20 : 1), and the orange band collected. The eluate (300 c.c.) was concentrated (to 4 c.c.), diluted with hot light petroleum (b. p. 40—60°; 10 c.c.), and cooled, the *dibenzoate* (32 mg.) being obtained as orange nodules. For analysis it was dried at 105°/0·2 mm. overnight (Found: C, 73·1; H, 4·3. $C_{44}H_{30}O_{10}$ requires C, 73·5; H, 4·2%). Light absorption in $CHCl_3$: max. at 489, 487, 430, 350, and 332 mμ (log ε 3·84, 3·83, 4·59, 4·03, 4·03, and 4·20 respectively). The compound

was very soluble in chloroform and ether, soluble in ethoxyethanol and cold benzene, slightly soluble in ethanol, and insoluble in light petroleum. It charred between 220° and 230° but had no definite m. p. The infra-red spectrum (Nujol mull) showed maxima at 1754, 1645, 1623, 1605, 1565, 1366, 1307, 1292, 1235, 1212, 1190, 1172, 1144, 1111, 1096, 1073, 1053, 1014, 962, 870, 840, 825, 800, 763, 719, 714, 704, and 696 cm.$^{-1}$.

Diacetyldihydroxyerythroaphin-fb.—Dipiperidinoerythroaphin-*fb* (53 mg.; from erythroaphin-*sl*; Part XII, *loc. cit.*) was dissolved in dry pyridine (7·5 c.c.) and acetylated by the addition, dropwise, of acetyl chloride (0·8 c.c.), the temperature being kept below 10°. After a further 15 min. at room temperature the solution was poured into ice-cold 3N-hydrochloric acid (50 c.c.) and after 3 min. the precipitated product was extracted into benzene (3 × 15 c.c.). The combined benzene extracts were washed with N-hydrochloric acid, then water, dried, and concentrated (to 5 c.c.). The orange-coloured solution, which contained a mixture of acetylated dipiperidinoerythroaphin-*fb* and acetylated dihydroxyerythroaphin-*fb*, was filtered and the warm filtrate diluted with light petroleum (10 c.c.; b. p. 40—60°). After slow cooling, orange crystals of *diacetyldihydroxyerythroaphin*-fb were obtained which were separated, washed, and dried (30 mg.) (Found, in a sample dried at 90°/10^{-3} mm. for 5 hr.: C, 65·4; H, 4·5. $C_{34}H_{26}O_{12}$ requires C, 65·2; H, 4·2%). Light absorption in $CHCl_3$: max. at 433, 349, 332, 320, 282, and 256 mμ (log ε 4·55, 3·93, 3·96, 4·08, 4·01, and 4·36 respectively). The infra-red spectrum, determined as a mull in Nujol, showed maxima at 3333, 1786, 1647, 1605, 1562, 1506, 1366, 1311, 1274, 1176, 1153, 1133, 1098, 1079, 1047, 980, 948, 935, 917, 904, 885, 864, 828, 803, 777, 760, 738, and 687 cm.$^{-1}$. Hydrolysis of the product in chloroform with 2N-sodium hydroxide at room temperature gave a red solution which showed the characteristic absorption maxima of dihydroxyerythroaphin-*fb* (hand spectroscope: max. at 596, 567, 526, and 450 mμ). The filtrate from the preparation of diacetyldihydroxyerythroaphin-*fb* was shaken with 50% hydrochloric acid which extracted the coloured material, and the acid extract was diluted with twice its volume of water and the product re-extracted into benzene The solution then showed the absorption maxima of dipiperidinoerythroaphin-*fb* (hand spectroscope: max. at 610, 574, 530, and *ca.* 450 mμ).

Irradiation of Tetra-acetyldihydroerythroaphin-sl. *Isolation of the* fb-*Isomer*.—Tetra-acetyldihydroerythroaphin-*sl* (500 mg.; $[\alpha]_D$ +35°; all rotations determined in C_6H_6) was dissolved in benzene (400 c.c.) and irradiated with ultra-violet light (λ 300—365 mμ) for 5 hr. The dark orange solution ($[\alpha]_D$ −48°) was concentrated (to 15 c.c.) *in vacuo* and chromatographed on a column of silica (9 × 5 cm.), elution being with benzene–acetone (20 : 1). The yellow-orange eluate was concentrated (to 50 c.c.), diluted with light petroleum (b. p. 40—60°; 200 c.c.), and kept for two days with exclusion of light. The crystalline product (268 mg.; $[\alpha]_D$ −170°) was separated and repeatedly recrystallised from benzene–light petroleum. The specific rotation of the product gradually approached that of the *fb*-isomer and after five such crystallisations the tetra-acetyl derivative (88 mg.) had $[\alpha]_D$ −435°. This product (73 mg.) was dissolved in chloroform (35 c.c.) and was shaken for 1 min. with a solution of aqueous sodium hydroxide (4 c.c. of 10%) in methanol (30 c.c.). The green suspension of the sodium salt of erythroaphin was acidified with dilute hydrochloric acid, and the red chloroform layer separated and washed with water and sodium hydrogen carbonate solution. The chloroform solution was dried and concentrated, and the erythroaphin (30 mg.) caused to crystallise (as dark red needles) by the addition of hot ethanol. It was separated, recrystallised from chloroform–ethanol, and then dried at 120°/1·5 mm. for 6 hr. The ultra-violet and infra-red absorption spectra of the product (25 mg.) were identical with those of erythroaphin-*fb*.

The combined mother-liquors from the benzene–light petroleum crystallisations of the irradiated product was kept for 4 weeks at room temperature in the dark. A second crop of crystals (50 mg.; $[\alpha]_D$ −230°) were removed and the mother-liquors concentrated (to 20 c.c.) and then diluted with light petroleum (90 c.c.). After several hours in the dark, a crystalline product (100 mg.) separated which had $[\alpha]_D$ +40°. Hydrolysis of this tetra-acetyl compound (75 mg.) by the procedure outlined above gave an erythroaphin (33 mg.) which recrystallised from chloroform–ethanol to give a product (25 mg.) as small dark red needles. It was dried at 100°/5 mm. for 12 hr. and the infra-red spectrum determined which was identical with that of erythroaphin-*sl*.

Irradiation of Tetra-acetyldihydroerythroaphin-fb. *Isolation of the* sl- *and the* trans-trans-*Isomer*.—Tetra-acetyldihydroerythroaphin-*fb* (685 mg.) was dissolved in purified dry benzene (800 c.c.) in an atmosphere of nitrogen, the yellow solution having $[\alpha]_D$ −565°. (All specific rotation values on the tetra-acetyldihydroerythroaphins were determined in benzene solution.) It was placed in a silica flask and irradiated with ultra-violet light (λ 300—365 mμ) for 18 hr.

after which the orange-red solution had $[\alpha]_D$ −300°. It was concentrated (to 100 c.c.) under reduced pressure, brought on a column (10 × 5 cm.) of silica, and eluted with benzene. The orange eluate was concentrated (to 100 c.c.), an equal volume of light petroleum added, and the solution kept for three days with exclusion of light. The orange crystals (495 mg.) formed were separated and the mother-liquors stored in a dark cupboard. The crystals were dissolved in benzene, giving a solution with $[\alpha]_D$ −470°, which was irradiated for 20 hr; the rotation was then $[\alpha]_D$ −180°. The purification was effected as before (giving 300 mg. of $[\alpha]_D$ −380°), the mother-liquors being set on one side and the crystals dissolved in benzene and again irradiated (to $[\alpha]_D$ −160°). Chromatography of the resulting solution gave a main fraction from which a crystalline product (235 mg.; $[\alpha]_D$ −355°) was obtained. Further crystallisations gave successive crops (185 mg. of $[\alpha]_D$ −480°; then 84 mg. of $[\alpha]_D$ −520°). The final product which formed orange nodules was identical with the original tetra-acetyldihydroerythroaphin-*fb*. Hydrolysis with methanolic sodium hydroxide as before gave erythroaphin-*fb* (26 mg. from 70 mg. of tetra-acetyl derivative), the infra-red spectrum of which was identical with that of an authentic specimen.

The four batches of benzene–light petroleum mother-liquors from the three irradiation experiments and the first of the subsequent crystallisations were combined and kept at room temperature for one week with exclusion of light. All operations from this stage onwards were carried out in subdued light or with total exclusion of light. The small amount of crystalline material which had separated was removed by filtration and the solution concentrated (to 100 c.c.) under reduced pressure. It was diluted with an equal volume of light petroleum and kept for three days. A further small amount of crystalline material was separated and the mother-liquors were concentrated (to 20 c.c.), diluted with light petroleum (80 c.c.), and kept in the dark overnight. The orange-brown crystals (32 mg.; A) were separated, and the mother-liquors concentrated (to 5 c.c.) and diluted with light petroleum (60 c.c.), more crystals (88 mg.; B) being obtained overnight. By further concentration (to 3 c.c.) of the mother-liquors, dilution with light petroleum (40 c.c.) and keeping in the dark for three weeks, another crop of heavy brown nodules (70 mg.; C) was obtained. Repetition of this process gave a small quantity of a dark brown powder (10 mg.; D) after one week. All of the fractions A, B, C, and D showed the typical visible-light absorption spectrum of tetra-acetyldihydro-erythroaphin with prominent bands at 467 and 502 mμ (hand-spectroscope).

Fraction A (30 mg.; $[\alpha]_D$ +20°) was hydrolysed with methanolic sodium hydroxide to give an erythroaphin (8 mg.), the infra-red spectrum of which showed the main characteristics of erythroaphin-*sl*.

Fraction C (70 mg.; $[\alpha]_D$ +380°) was more soluble in benzene than the *fb*- or *sl*-isomer. It was hydrolysed as before with methanolic sodium hydroxide, and the erythroaphin purified by partition between chloroform and sulphuric acid of various strengths (Part III, *J.*, 1950, 485). After crystallisation of the product from chloroform–ethanol, the trans-trans-*isomer* was obtained as dark red crystals (22 mg.), m. p. 240—242° with previous darkening, $[\alpha]$ +325° (in $CHCl_3$; white light) (Found: C, 70·5; H, 4·0. $C_{30}H_{22}O_8$ requires C, 70·6; H, 4·3%). Light absorption in $CHCl_3$: max. at 589, 564, 523, 486, 448, 422, and 254 mμ (log ε 3·80, 4·17, 3·99, 3·70, 4·46, 4·35, and 4·46 respectively). The infra-red spectrum determined on a mull in Nujol showed maxima at 667, 691, 722, 741, 763, 789, 813, 829, 855, 870, 964, 1000, 1047, 1076, 1111, 1164, 1183, 1212, 1255, 1279, 1346, 1587, and 1626 cm.$^{-1}$.

Fraction C (88 mg.; $[\alpha]_D$ +185°) was converted into the corresponding erythroaphin which was purified by the sulphuric acid method and crystallised from chloroform–ethanol as before. The product (17 mg.; $[\alpha]_D$ +190°) appeared from the infra-red spectrum to be a mixture of the *sl*- and the *trans-trans*-isomer.

Penta-acetyldihydrohydroxyerythroaphin-fb.—Erythroaphin-*fb* (100 mg.) was treated with acetic anhydride and perchloric acid by the method described in Part VII (*loc. cit.*). The ethereal extract of the product was washed with 1% hydrochloric acid and then with 0·5% aqueous sodium hydroxide and dried. After removal of solvent, the residue was dissolved in benzene (10 c.c.), brought on a column of silica (9 × 3 cm.), and eluted with benzene. The deep yellow benzene eluate was concentrated to small bulk (1 c.c.), diluted with light petroleum, and kept overnight. The precipitated brown solid (22 mg.) was separated, redissolved in benzene (0·5 c.c.), and again obtained as a non-crystalline powder by addition of light petroleum (3 c.c.). For analysis it was dried at 120°/0·5 mm. for 8 hr. (Found: C, 64·8; H, 4·3. $C_{40}H_{34}O_{14}$ requires C, 65·0; H, 4·6%). This *compound* was very soluble in benzene, acetone, chloroform, ethanol, and ethyl acetate.

In another experiment, erythroaphin-*fb* (50 mg.) was dissolved in "AnalaR" acetic

anhydride (8 c.c.) containing perchloric acid (1 drop of 60%) and kept at 0° for 24 hr. The reaction, as well as the subsequent purification, were carried out with exclusion of light. Excess of anhydride was hydrolysed by shaking the mixture with saturated aqueous sodium acetate (32 c.c.) at 0° for 30 min. The product was extracted with ether (50 c.c.), and the extract washed successively with 1% hydrochloric acid (3 × 50 c.c.), saturated sodium hydrogen carbonate solution (2 × 50 c.c.), 1% sodium hydroxide solution (4 × 30 c.c.), alkaline sodium dithionite solution (2 × 30 c.c.), and water (2 × 50 c.c.), and the resulting bright yellow ethereal solution was dried. The optical rotation was measured ($[\alpha]_D$ −230°) and the solution in the polarimeter tube irradiated with a tungsten lamp. After 12 hr. the rotation had fallen to $[\alpha]_D$ −130° and after 24 hr. to $[\alpha]_D$ −30°. The concentration (45 mg./100 c.c.) was estimated by measurement of the optical density at 496 mμ in chloroform solution.

In another experiment penta-acetyldihydrohydroxyerythroaphin-*sl* was prepared, also with exclusion of light as described above. The optical rotation of the ethereal solution (26 mg./100 c.c.) which initially was $[\alpha]_D$ −40° changed to $[\alpha]_D$ −30° after irradiation with a tungsten lamp for 24 hr. The combined irradiated solutions were evaporated and the residue was dissolved in benzene and chromatographed on a column of silica. The benzene eluate showed maxima at 490, 457, 430, and 407 mμ, as did the original solutions before irradiation. Evaporation of the benzene solution caused extensive decomposition.

Hexa-acetyldihydrodihydroxyerythroaphin.—Both hydroxyerythroaphin-*fb* and -*sl* were treated with acetic anhydride and perchloric acid with exclusion of light by the method described in the previous paragraphs. Extensive decomposition occurred during the reaction but the ethereal solutions of the products were irradiated as before with a tungsten lamp. The optical rotations of both solutions were zero both before and after irradiation. Attempted isolation of the product merely resulted in decomposition.

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