## 14. *Colouring Matters of the Aphididæ. Part XXI.*[1] *Nuclear Magnetic Resonance Evidence for the Structures of the Erythroaphins and their Derivatives.*

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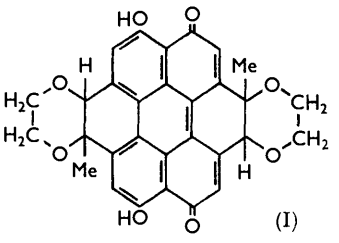
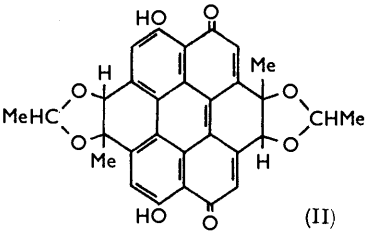
Nuclear magnetic resonance spectra of the erythroaphins-*fb* and -*sl* and of the dihydrotetramethylerythroaphins are inconsistent with structures of these molecules that contain substituted dioxan or dioxalan rings. The spectra led to consideration of structures in which each methyl group has an adjacent CH bond, and hence to the type of structure that includes formula (III), now proposed on chemical and spectroscopic grounds. The spectra of the substituted erythroaphin derivatives show that halogen substituents replace hydrogen atoms in aromatic CH bonds, but that nitrogen-containing or hydroxyl substituents replace CH bonds in the aliphatic parts of the molecule.

In this Paper an account is given of the contributions made by nuclear magnetic resonance spectroscopy to the elucidation of the structural formulæ of the erythroaphins and their derivatives. The spectra of the hydrogen nuclei in the erythroaphins themselves and their dihydrotetramethyl derivatives have been found to be inconsistent with structural formulæ (I and II) that had been put forward in an earlier paper of the series.[2] This led to consideration of an alternative type of formula with the partially saturated rings attached at six positions on the perylenequinone nucleus. The spectra provide detailed evidence




(I)

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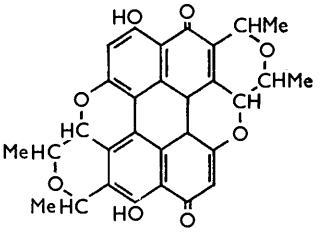


(III)

in favour of the particular formula (III) of this type which best accords with chemical evidence.[3] Spectroscopic evidence on the stereochemical aspects of the formulæ of the erythroaphins and the protoaphins[4] is given in the following paper.

*Spectra of the Erythroaphins and the Dihydrotetramethylerythroaphins (General Aspects).*—In the spectrum of erythroaphin-*fb* there is only a single resonance from the CH groups that form part of the perylenequinone nucleus. This may be the result either of a centrosymmetrical formula for the dihydroxyperylenequinone nucleus, or of rapid hydrogen-transfer between the hydroxyl groups in alternative *peri*-positions of a non-centrosymmetrical perylenequinone ring. The resonances of such CH groups are expected in the general region of $\tau$ values from 2·5 to 3·5[5] and are found at $\tau$ 3·39 for *fb* and $\tau$ 3·35 and 3·42 for *sl* (two lines of equal intensity) in saturated solutions in $CDCl_3$.

The following distribution of intensities of hydrogen resonances (based on the total

[1] Part XX, Calderbank, Cameron, Cromartie, Hamied, Haslam, Kingston, Todd, and Watkins, preceding paper.
[2] Part XIII, Brown, Calderbank, Johnson, Joshi, Quayle, and Todd, *J.*, 1955, 959.
[3] Part XVIII, Cameron, Cromartie, Hamied, Scott, and Todd, *J.*, 1964, 62.
[4] Part XVII, Cameron, Cromartie, Kingston, and Todd, *J.*, 1964, 51.
[5] Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, pp. 52, 59, 60, 63.

from the two sides of the molecule) is expected from the remaining CH bonds in formulæ I and II:

| | CH-O ($\tau \sim 4{\cdot}0$—$6{\cdot}5$) [5] | $CH_3$-C ($\tau \sim 8{\cdot}0$—$9{\cdot}5$) [5] |
|---|---|---|
| Formula I ..... | 10 | 6 |
| Formula II ..... | 4 | 12 |

It was clear from the outset, both for erythroaphin-*fb* and for -*sl*, that most intensity in the proton spectra was concentrated in the $\tau$ range 8·2—8·9 and hence that formula (I) was untenable. In the case of erythroaphin-*sl* a well-defined spectrum was obtained (Fig. 1B) which was complex because of a somewhat different pattern of chemical shifts and coupling constants originating in the two sides of the molecule. In the case of the more symmetrical *fb* isomer a simpler spectrum (Fig. 1A) was obtained, as expected, in the region of $CH_3$–C resonances, but limited solubility precluded any detailed study of the

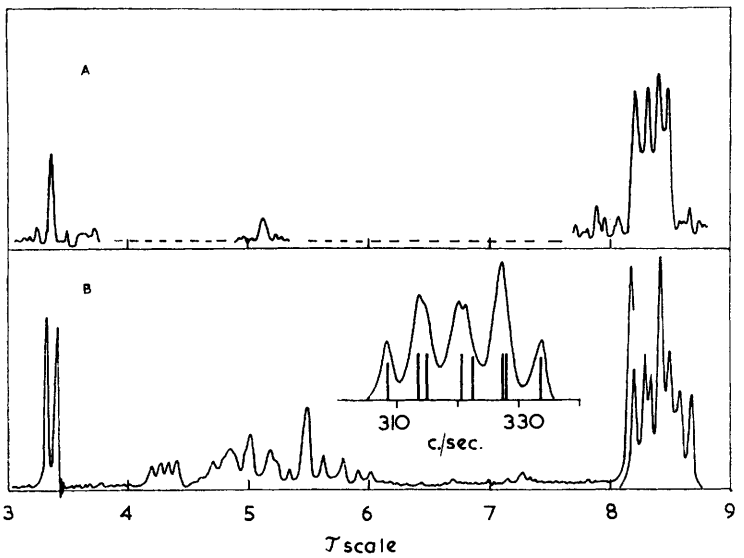


FIG. 1. The nuclear magnetic resonance spectra at $\tau$ 3—9 of the erythroaphins: (A) erythroaphin-*fb* at 40 Mc./sec., and (B) erythroaphin-*sl* at 40 Mc./sec.; details of the methyl group resonances at 56·445 Mc./sec. are shown as an inset with the calculated line-positions in c./sec. from the $CHCl_3$ resonance indicated as vertical lines.

$\tau$ *ca.* 5·5 region. It was also clear that the pattern of fine-structure lines obtained in the C–$CH_3$ region was difficult to reconcile in detail with formula (II). Thus for the *fb* derivative, where chemical evidence indicates that the two sides of the molecule are likely to be identical,[3,6] three peaks are to be expected in the C–$CH_3$ region for the dioxalan formula, one of relative strength 6 corresponding to the chemically equivalent quaternary $CH_3$ groups, and two of strength 3 each from the equivalent tertiary $CH_3$ groups, each resonance having been split into a doublet (spacing 6—7 c./sec.) by spin–spin coupling with the adjacent CH hydrogen.[7] For the *sl* isomer, with different, geometrically isomeric sides of the molecule,[3,6] two lines of strength 3, and four (in two pairs) of strength $1\frac{1}{2}$, are to be expected. In fact, the *fb* spectrum gives four lines of equal intensity, and the *sl* spectrum six of equal intensity and only one of double intensity in the C–$CH_3$ region. (Figs. 1A and 1B.)

The most likely explanation on spectroscopic grounds seemed to be that every methyl

[6] Part XIV, Brown, Calderbank, Johnson, Quayle, and Todd, *J.*, 1955, 1144.
[7] Jackman, *op. cit.*, p. 87.

resonance is a doublet caused by coupling with an adjacent CH, and that the one line of double intensity in the *sl* spectrum is caused by chance overlap of two of the expected lines. A careful comparative analysis of 40 and 56·445 Mc./sec. spectra from the same solutions of erythroaphin-*fb* and -*sl* showed this to be the case. The observed line positions at 40 Mc./sec. and the calculated spectrum at 56·445 Mc./sec., compared with the observed one, are listed in Table 1. In the case of erythroaphin-*fb*, only the assignment of lines listed gave good agreement with the 56·445 Mc./sec. spectrum. For erythroaphin-*sl* the assignment of the 40 Mc./sec. spectrum as four methyl doublets again gave best agreement

TABLE 1.

Observed and calculated line positions (in c./sec. from the chloroform resonance) of the methyl resonances in the 40 and 56·445 Mc./sec. spectra of erythroaphins-*fb* and *sl*.

*Erythroaphin*-fb *

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 40 Mc./sec. (obs.) ..... | 219·7 ± 0·3 | 223·0 ± 0·3 | 226·7 ± 0·35 | 229·3 ± 0·4 |
| 56·445 Mc./sec. (obs.) ..... | 311·6 ± 0·15 | 316·7 ± 0·25 | 318·4 ± 0·25 | 322·1 ± 0·3 |
| 56·445 Mc./sec. (calc.) † ... | 311·4 | 316·0 | 318·4 | 322·4 |

* Errors are standard deviations. † Best fit of observed and calculated spectra based on the assignment of lines 1 and 3, and 2 and 4 of the 40 Mc./sec. spectra to the doublets from the two methyl groups.

*Erythroaphin*-sl ‡

| | 1 | 2 | 3 | 4 § |
|---|---|---|---|---|
| 40 Mc./sec. (obs.) ..... | 217·5 ± 0·2 | 221·2 ± 0·3 | 224·0 ± 0·3 | 227·9 ± 0·5 |
| 56·445 Mc./sec. (obs.) ..... | 308·1 ± 0·3 | 313·5 ± 0·4 | 314·1 ± 0·6 | 320·2 ± 0·4 |
| 56·445 Mc./sec. (calc.) ¶ ... | 308·2 | 313·4 | 314·8 | ~320·3 |

| | 5 § | 6 § | 7 § | 8 |
|---|---|---|---|---|
| 40 Mc./sec. (obs.) ..... | 227·9 ± 0·5 | 231·4 ± 0·2 | 232·9 ± 0·3 | 237·8 ± 0·2 |
| 56·445 Mc./sec. (obs.) ..... | 321·4 ± 0·6 | 327·4 ± 0·6 | 327·4 ± 0·6 | 333·6 ± 0·4 |
| 56·445 Mc./sec. (calc.) ¶ ... | ~322·5 | 327·5 | 327·7 | 334·2 |

‡ Estimated probable errors. § Lines 4 and 5 closely overlap at 40 Mc./sec.; lines 6 and 7 overlap at 56·445 Mc./sec. ¶ Based on the pairing of lines 1 and 3, 2 and 4, 5 and 7, and 6 and 8 in the 40 Mc./sec. spectrum.

at 56·445 Mc./sec.; the observed and the calculated spectrum of erythroaphin-*sl* at the latter frequency are compared in Fig. 1B.

The complexity of the spectrum of erythroaphin-*sl* in the $\tau$ 4—6·5 region was also difficult to account for on formula (II), which predicts two sharp single peaks from the chemically non-equivalent lone CH bonds on the two sides of the molecule, plus two regular quartets from the CH resonances of the CH–$CH_3$ groups. This region of the spectrum of erythroaphin-*fb* was too weak for analysis owing to limited solubility of the sample in chloroform or other solvents, and we therefore studied the spectra of the more soluble dihydrotetramethyl and tetra-acetyldihydro-derivatives of the erythroaphins.

The spectra of the three dihydrotetramethylerythroaphins -*fb*, -*sl*, and -*tt* [8] (the last being the other symmetrical isomer obtained by ultraviolet irradiation of the dihydrotetramethylerythroaphin-*sl* [6]) are shown in Fig. 2. Although some difficulty was caused by slow decomposition of these compounds in solution, the main features of the spectra in the C–$CH_3$ region were consistent with those of the erythroaphins themselves. For the *sl* and *fb* compounds there is more overlapping of lines than in the spectra of the erythroaphins, but the spectrum of the *tt* derivative is a slightly distorted version of the expected 4-line pattern. It is thought that the distortion is caused by the presence of a small amount of impurity.

As was hoped, these spectra in the $\tau$ ~4—6·5 region were better defined than those of the parent compounds. Also it was found that there were two quite separate resonances

[8] Part XIX, Cameron, Cromartie, Hamied, Joshi, Scott, and Todd, *J.*, 1964, 72.

TABLE 2.

Chemical shifts and coupling constants in the spectra of the erythroaphins and their derivatives. Chemical shifts in p.p.m. on the $\tau$ scale ($SiMe_4$, $\tau$ = 10·00); coupling constants in c./sec. (in parentheses).

| No. | Compound | Arom. CH | | O–CH<C | | C |
|---|---|---|---|---|---|---|
| 1 | Erythroaphin-*fb* | 3·39 | — | V. weak spectrum | | — |
| 2 | Diacetamido EA-*fb* | 3·23 | — | 4·59(Q) (7) | — | 5·47(Q) (6·3) |
| 3 | Dipiperidino EA-*fb* | 3·23 | — | 4·72(Q) (6·5) | — | 4·98(Q) (6·3) |
| 4 | Diaminodichloro EA-*fb* | — | — | 4·62(Q) (7) | — | 5·37(Q) (6·5) |
| 5 | Dichlorodihydroxy EA-*fb* | trace | — | 4·87(Q) (~6·5) | — | 5·51(Q) (~6) |
| 6 | Dihydrotetramethyl EA-*fb* | 3·57 | — | 4·79(Q) (6·7) | 5·07(D) (9·6) | 5·69(D × Q) (~10 and 6·2) |
| 7 | Dichlorodihydrotetramethyl EA-*fb* | — | — | Poorly resolved | | |
| 8 | Tetra-acetyldihydro EA-*fb* | ~3·2 | — | P.R. | ~4·86(D) (~9) | P.R. |
| 9 | Tetra-acetyldichlorodihydro EA-*fb* | — | — | P.R. | ~4·81(D) (~9) | P.R. |
| 10 | Erythroaphin-sl † | 3·35, 3·42 | 4·29(D × D) (2·8, 5·8) | | Complex | |
| 11 | Dihydrotetramethyl EA-*sl* | 3·49 | ~4·38(D) (~6) | | Complex | |
| 12 | Dichlorodihydrotetramethyl EA-*sl* | — | 4·31(D) (~5) | | Complex | |
| 13 | Tetra-acetyldihydro EA-*sl* | 3·17, 3·24 | 4·28(D) (~6) | P.R. | ~5·0(D) (~10) | P.R. |
| 14 | Dihydrotetramethyl EA-*tt* | 3·54 | 4·33(D) (~6) | 4·90(Q?) (~6) | — | 5·4(D × Q?) (~6 and 6) |

| No. | C–$CH_3$ | | | | Others ($\tau$ only) | Fig. and ref. substance * | |
|---|---|---|---|---|---|---|---|
| 1 | 8·33(D) (7·0) | — | 8·40(D) (6·3) | — | — | 1A | $CHCl_3$ |
| 2 | 8·30(D) (7) | — | 8·40(D) (6·5) | — | 8·17 (CO·$CH_3$) 3·52 (CO·NH) | 3B | $SiMe_4$ |
| 3 | 8·23(D) (6·5) | — | 8·85(D) (6·5) | — | 9·6 (C–$CH_2$–C) 7·2 (C–$CH_2$–N) | 3A | $SiMe_4$ |
| 4 | 8·32(D) (7) | — | 8·41(D) (6·5) | — | 3·9 ($NH_2$) | | $SiMe_4$ |
| 5 | ~8·4(D) | — | ~8·5(D) | — | — | | $SiMe_4$ |
| 6 | 8·36(D) (~7) | — | 8·48(D) (~6) | — | 6·26, 6·07 ($OCH_3$) 8·77 (impurity) | 2A | $SiMe_4$ |
| 7 | ~8·33(D) P.R. | — | ~8·41(D) P.R. | — | 6·17, 6·12 ($OCH_3$) | | $CHCl_3$ |
| 8 | ~8·49(D) P.R. | — | ~8·59(D) P.R. | — | 7·43 (O·CO·$CH_3$) | | $CHCl_3$ |
| 9 | ~8·44(D) P.R. | — | ~8·44(D) P.R. | — | 7·55 (O·CO·$CH_3$) | | $CHCl_3$ |
| 10 | 8·25(D) (6·5) | 8·34 (~6·7) | 8·48 (~6) | 8·59 (~6·4) | −4·98, −4·72 (OH) | 1B | $SiMe_4$ |
| 11 | ~8·36(D) (~6) | ~8·36(D) (~6) | ~8·49(D) (~6) | ~8·62(D) (~7) | 6·24, 6·18 6·01 ($OCH_3$) | 2B | $SiMe_4$ |
| 12 | 8·30(D) (~6) | | Complex | | ~6·1 (overlapping) ($OCH_3$) | | $CHCl_3$ |
| 13 | ~8·42(D) (~6) | | Complex | | 7·60 (O·CO·$CH_3$) | | $CHCl_3$ |
| 14 | — | 8·39(D) (~6·5) | — | 8·69 (7·2) | 6·25, 6·0 ($OCH_3$) | 2C | $CHCl_3$ |

EA = erythroaphin; (D) = doublet; (Q) quartet; (D × D) = doublet of doublets; (D × Q) = doublet of quartets; P.R. = poorly resolved spectrum.

* The values for the chemical shifts are likely to be somewhat more accurate for those compounds for which the internal standard tetramethylsilane, rather than the solvent $CHCl_3$, resonance was used for reference. † Chemical shifts are somewhat concentration-dependent; values given are for saturated solution.

of apparently equal intensity for the additional $CH_3O$ groups near $\tau$ 6·05 and 6·25 (Fig. 2 and Table 2); for the *sl* compound, the high-field one of these is further split into a narrow doublet. The analogous tetra-acetyldihydroerythroaphins [9,10] gave only a single $O{\cdot}CO{\cdot}CH_3$ resonance in the region $\tau$ 7·4—7·6 for both the *fb* and the *sl* derivatives investigated. However, the marked chemical shift between the resonances of the two pairs of methoxyl groups in the dihydrotetramethylerythroaphins was again very difficult to reconcile with

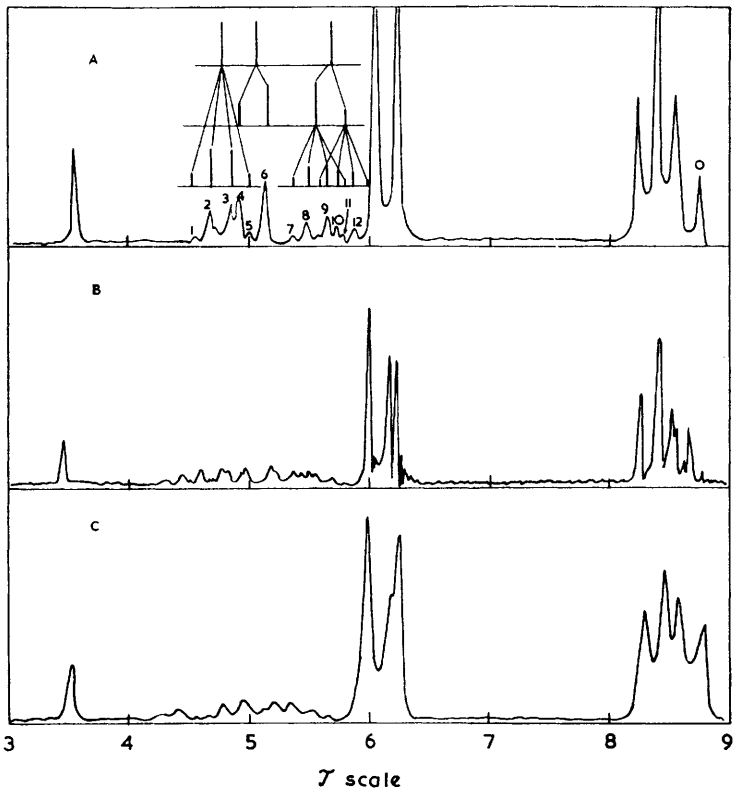


FIG. 2. The nuclear magnetic resonance spectra at 40 Mc./sec. of the dihydrotetramethyl derivatives of (A) erythroaphin-*fb* (with the interpretation indicated in the $\tau$ 4—6·5 region), (B) erythroaphin-*sl*, and (C) erythroaphin-*tt*. o denotes an impurity resonance.

the expected formulæ of the molecules derived from structure (II), where each methoxyl group is similarly flanked by an " aromatic " CH bond.

Although no reasonable alternative to formula (II) could be suggested on chemical grounds at this stage, it was clear that a new one would have to be found to accommodate the spectroscopic results. It seemed possible to fill these requirements, in particular the spectroscopic evidence for two $CH_3$–CH groups on each side of an erythroaphin molecule, only by adopting one of a number of alternative formulæ in which the partially saturated rings occupy three of the four substitution positions on each side of the aromatic skeleton (six out of eight positions in the complete molecule). Formula (III), finally adopted on chemical and spectroscopic grounds, was one of the possibilities envisaged.

[9] Part II, Human, Johnson, MacDonald, and Todd, *J.*, 1950, 477.
[10] Part III, Duewell, Johnson, MacDonald, and Todd, *J.*, 1950, 485.

Formulæ of this general type had previously been considered in relation to the chemical evidence, but put aside on the assumption that tetrasubstituted erythroaphin derivatives, such as dibromodipiperidinoerythroaphin, were formed by substitution at four aromatic-quinonoid CH groups. The new formulæ allowed the presence of only two " aromatic " CH bonds, but it had two additional spectroscopic advantages. First, it accounted directly for the rather different chemical shifts of the two pairs of methoxyl groups in the dihydrotetramethylerythroaphins and, secondly, the additional pair of saturated CH bonds (one on either side) helps to account for the complexity in the $\tau$ 4—6·5 region of the spectrum of erythroaphin-*sl*.

Finally, an intensity-integrated spectrum of erythroaphin-*sl*, subsequently obtained, confirmed the finding that only two " aromatic " CH bonds are present.

*Spectra of Di- and Tetra-substituted Erythroaphin Derivatives, and the Location of Substituent Groups.*—The first confirmation of formulæ similar to (III) came from the spectra

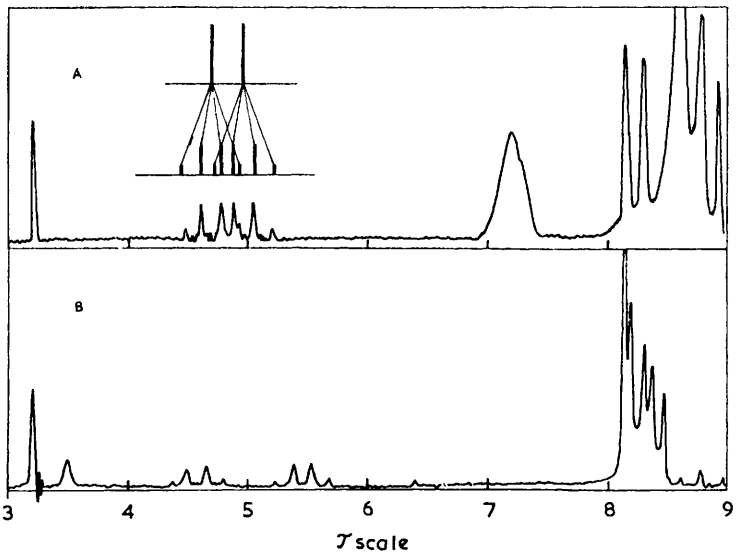


FIG. 3. The nuclear magnetic resonance spectra at 40 Mc./sec. of (A) dipiperidino-erythroaphin-*fb* and (B) diacetamidoerythroaphin-*fb*.

of the dichlorodihydrotetramethylerythroaphins-*fb* and -*sl* [8] and dichlorotetra-acetyldihydroerythroaphin-*fb*,[8] which showed complete absence of aromatic CH resonances. It is clear, therefore, that the halogen substituents replace aromatic hydrogen atoms and that there are only two of the latter. The dichloro-compounds of the erythroaphins themselves [11] are insufficiently soluble to give good spectra.

Dipiperidino-[11] and diacetamido-erythroaphin-*fb* [8] show aromatic CH peaks of apparently undiminished intensity, so that these substituents must be located in the aliphatic parts of the molecules. Direct confirmation was obtained from a study of the spectra of the last two compounds (Figs. 3A and B) and of diaminodichloroerythroaphin-*fb* [8] in the $\tau$ 4—6·5 region. These spectra consist of two symmetrical quartet resonances (internal relative intensities 1 : 3 : 3 : 1) as expected from the lone CH bonds in $CH{-}CH_3$ groups. On the other hand, the spectrum of dihydrotetramethylerythroaphin-*fb* itself (expected to have aliphatic groupings identical with those in erythroaphin-*fb*) is considerably more complicated in this region (Fig. 2A) and, as we shall show below, can be analysed in detail

[11] Part VII, Brown, Johnson, MacDonald, Quayle, and Todd, *J.*, 1952, 4928.

as consisting of one isolated quartet resonance plus the AB part of an $ABX_3$ spectrum [12] (Fig. 2A) corresponding to the grouping $CH_A$–$CH_B$–$CH_3$ shown in (III). Clearly the acetamido- and piperidino-substituents replace $H_A$.

Similar double-quartet spectra to those obtained with the diacetamido- and dipiperidino-compounds were subsequently obtained from dichlorodihydroxyerythroaphin-*fb*,[8] implying that the hydroxyl groups are placed at the same positions in the aliphatic part of the molecule.

*More Detailed Discussion of the Spectra in Relation to Formula* (III).—The region of the spectrum of dihydrotetramethylerythroaphin-*fb* between $\tau$ 4 and $\tau$ 6·5 is particularly well resolved (Fig. 2A) and can be analysed in detail. Lines 1, 2, 3, and 5 comprise a symmetrical quartet ($\tau$ 4·79, J = 6·7 c./sec.) analogous to those found close to this position in the double-quartet spectra of the substituted erythroaphins discussed above. With the exception of the dipiperidino-derivative, to be discussed below, the latter spectra show the second quartet near $\tau$ 5·5, and a more complicated pattern of lines occurs near this position in the spectrum of dihydrotetramethylerythroaphin-*fb*. The latter spectrum has two strong additional lines (4 and 6) which must be assigned to the aliphatic hydrogen atom replaced by the nitrogen-containing substituents. The resonance is clearly split by coupling with the hydrogen nucleus of an immediately adjacent CH bond ($J$ 9·6 c./sec.), which in turn must be part of one of the CH–$CH_3$ groups. The middle CH bond should therefore give a resonance consisting of a doublet of quartets and it can be seen that the complex spectrum in the region of $\tau$ 5·5 is of this type with lines 7, 8, 9, and 11 constituting one quartet, and lines 10 and 12 as the central strong lines of the second quartet. The unequal intensities of lines 4 and 6, and of the two quartets, are caused by the small chemical shift between the resonances of the adjacent hydrogen atoms so that the spectrum is of the $ABX_3$ type mentioned above from the $CH_A$–$CH_B$–$CH_3$ group. For $H_A$, $\tau$ = 5·07 ($J$ = 9·6 c./sec.), for $H_B$ $\tau$ = 5·69 ($J$ ~10 and 6·2 c./sec.). The $CH_3$ group gives one of the doublet resonances in the $\tau$ 8—9 region, and that at $\tau$ 8·48 is indicated as the appropriate one from the magnitude of the doublet splitting ($J$ ~6 c./sec.). By difference the quartet at $\tau$ 4·79 and the doublet at $\tau$ 8·36 are to be assigned to the other CH–$CH_3$ group in formula (III).

The linking together of the high-field CH quartet with the high-field methyl doublet proposed above is confirmed by the magnitudes of the mutual coupling constants listed in Table 2 from the spectra of other erythroaphin-*fb* derivatives, and also by the fact that it is these two resonances which exhibit a notable change in chemical shift on introduction of the bulky piperidino-substituent at the adjacent carbon atom.

Finally, on the basis of formula (III) it is seen that, as expected, it is the low-field CH–$CH_3$ resonances that are associated with the group directly attached to the aromatic nucleus (probably because of the operation of ring-currents in the latter [13]), and for the $CH_A$–$CH_B$–$CH_3$ group $H_A$, which is attached to the aromatic ring, is again at lower field than $H_B$ which is not. We conclude that the spectrum of dihydrotetramethylerythroaphin-*fb* gives excellent detailed agreement with expectations based on formula (III) for the parent erythroaphin.

A comparison of the spectrum of dihydrotetramethylerythroaphin-*tt* (the other symmetrical isomer) with that of the *fb* isomer shows that the most notable changes involve the resonance of $H_A$ (which in the *tt* isomer has moved downfield to $\tau$ 4·33 in comparison with $\tau$ 5·07 for the *fb* isomer), and of one of the methyl groups which has moved to the notably higher value of 8·69. Unfortunately the rather poorly resolved spectrum in the $\tau$ 4—6·5 region for the *tt* isomer does not allow sufficiently precise determination of the mutual coupling constants to decide which methyl group has the high-field resonance.

The spectra of erythroaphin-*sl* and its derivatives are all rather complex because of

[12] Pople, Schneider, and Bernstein, " High Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, 1959, p. 98 and Ch. 6; also Jackman, *op. cit.*, p. 89.
[13] Pople, Schneider, and Bernstein, *op. cit.*, p. 181; Jackman, *op. cit.*, p. 18.

overlapping of the resonances from the two different sides of the molecule and therefore cannot be analysed in detail. However, when they can be partially resolved, as in the $CH_3$ region for erythroaphin-*sl* and in the $\tau$ 4—6·5 region in tetra-acetyldihydroerythroaphin-*sl*, the separate resonances are found close to the positions expected from the spectra of dihydrotetramethylerythroaphin-*fb* and -*tt*. This confirms the view that the two sides of the *sl* isomer are related as expected to the structures of these isomers.[6] Erythroaphin-*sl* and its tetra-acetyldihydro-derivative show the expected pair of resonances from the aromatic CH bonds; in the dihydrotetramethyl derivative these are not separately resolved because of a smaller chemical shift, but the resonance observed is broad. A unique phenomenon is shown by the resonance of erythroaphin-*sl* at $\tau$ 4·29 which consists of a doublet of doublets ($J$ 5·8 and 2·8 c./sec.). The origin of this additional small splitting and the cause of the different $J_{AB}$ coupling constants (~10 c./sec. for *fb* and ~6 c./sec. for *tt*) associated with the two types of molecule will be discussed in the following paper.

## Experimental

Nuclear magnetic resonance spectra of the hydrogen nuclei were obtained at 40 Mc./sec. with initially a Varian 4300B spectrometer and 12″ electromagnet with flux stabilisation and sample spinning. The positions of resonances in these spectra relative to the chloroform solvent peak, or to that of tetramethylsilane used as an internal reference, were measured by using sidebands generated by a Muirhead-Wigan D695A decade oscillator. Later spectra were run on a Perkin-Elmer spectrometer with a thermostat-controlled permanent magnet. In this case the drift in the magnetic field was less than 2 c./sec. per hr., and peak positions as indicated on pre-printed charts were corrected for drifts in the tetramethylsilane resonances recorded at the beginning and the end of each spectrum.

Chemical shifts were measured on the $\tau$ scale [5,14] in parts per million [$\tau(SiMe_4) = 10{\cdot}00$] by measurement from the chloroform resonance in the earlier spectra [$\tau(CHCl_3) = 2{\cdot}75$] and directly from the tetramethylsilane resonance in the more recent spectra. Sharp lines were measured to an accuracy of $\pm 0{\cdot}05$ p.p.m. ($\pm 2$ c./sec.) and usually better. Relative spacings of closely spaced sharp lines are correct to $\pm 0{\cdot}5$ c./sec. or better. Spectra were obtained from solutions (usually saturated) in chloroform or deuteriochloroform.

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