The structure of the colourant/pigment, carmine derived from carminic acid

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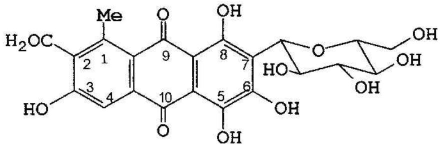
The colourant/pigment carmine synthesised from carminic acid and calcium and aluminium acetates has been examined chemically and spectroscopically to determine its structure. As with alizarin, it appears probable that the material is a tetramer but composed of four carminic acid units with aluminium involved with the 5,6-dioxy groups in five-membered rings and calcium chelated with the 10-carbonyl group of the anthraquinone system.

Keywords: alizarin, carminic acid, carmine, mass spectrometry, orbital negative analysis, ^{13} C solid state spectra

Carminic acid[1-3] (1) is obtained primarily in South America from the natural product cochineal which has been known since antiquity and which substantially displaced the use of the related material kermesic (its aglycone) acid in Europe. It is employed as a pink/red colourant in drinks (for example Campari) and as carmine, a red pigment, in the form of the sodium salt after complexation with aluminium (crimson lake, for watercolours) and as a less soluble red/purple pigment containing both calcium and aluminium. Both carminic acid and carmine have a variety of edible and non-edible applications. The study of the structure of carmine was influenced by the extensive work of many investigators on the metal complexes of alizarin, (2,3-dihydroxyanthra-9,10-quinone), culminating in the studies of Kiel and Heertjes[4] following earlier work.[5,6] Before the revision of the structure of carminic acid, suggestions on the structure of carmine were proposed[7] based on the work on alizarinates[4] and on the revised structure for carminic acid in which the carboxyl group was moved from the 4- to the 2-position. A similar view was taken by Meloan et al.[8] and models constructed which indicated the improbability of carminic acid having a 4-carboxyl group. No further studies have been reported on carmine and its structure has not been established although in the alizarinates more recent work[9] based on X-ray structural investigation has suggested that the calcium rather than the aluminium ion is the anthraquinone chelatant.

In work on carmine, apart from occasional IR studies, spectroscopic methods, proton NMR, solid state ^{13}C spectroscopy and mass spectrometry have not been employed. In the present work, these techniques have been invoked to examine its structure and significant progress achieved in negative ion mass spectrometry and modelling with related systems.



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Experimental

Spectroscopy: Proton NMR was effected on a CFT 20 instrument at Brunel University. Solid state NMR was carried out by Dr D. Apperley at the ERSRC centre, Industrial Chemical Laboratory, University of Durham, mass spectrometry at the EPSRC Mass Spectrometry Centre, University of Swansea, Wales and accurate mass measurements by orbitrap negative ion analysis.

Chromatography: TLC was performed on microscope slides coated with silica gel G_{254} (0.25 mm) and visualised under UV light or by staining with iodine.

Elemental analyses: By NRM Ltd, Bracknell, Berks.

Materials: Chemicals were obtained from the Aldrich Chemical Co. Carminic acid was also supplied by Christian Hansen, Denmark and European Colour Ltd. Plumstead, London.

Synthesis of colourants/pigments

Preparative experiments for obtaining carmine were initially modelled on those used for calcium aluminium alizarinate.[4]

Al, Ca, alizarinate: To alizarin (2.40 g, 0.01 m) in water (92 cm^3) containing 3M sodium hydroxide (8.2 cm^3, 0.0246 m), calcium acetate (0.91 g, 0.00575 m) and basic aluminium acetate (1.0346 g, 0.00862 m) each in hot water (10 cm^3) were added and after adjustment to pH 5.5 with 3M hydrochloric acid (1 cm^3), the mixture was diluted with water to 200 cm^3 and refluxed for 2 hours. The hot mixture was then filtered and the solid washed with water to remove electrolyte. The product was dried in a vacuum desiccator to give 2.7615 g (91.4%) containing 9.5% water, m.p. > 300°C; Found, C, 54.62; H, 2.85; Al, 3.51 requires for $C_{28}H_{12}O_8AlCa$; C, 54.72; H, 3.094; Al, 4.40%.

Al, Ca, carminate: To carminic acid (4.9205 g, 0.01 m) in water (22 cm^3) containing 3M sodium hydroxide (8.30 cm^3), calcium acetate (1.0102 g, 0.00639 m) and basic aluminium acetate (1.0242 g, 0.0085 m) each in hot water (10 cm^3) were added. The mixture was diluted with water to 200 cm^3 and refluxed for 1.5 h. The cooled material was acidified to pH 3 and sodium chloride added to precipitate the product, which after keeping at ambient temperature to complete the process, was filtered. The solid was washed with water to remove salts and dried in a vacuum desiccator (yield 3.3531 g, 68%).

The process was repeated with a purer source of carminic acid and the salting-out step omitted to avoid contamination with sodium chloride, although the product proved less easy to isolate. This sample was used in spectroscopic work. A smaller yield of dark red product was obtained (ca 20%, which increased with time) and the product was dried in a vacuum desiccator; Found, C, 47.91; H, 3.86; Al, 2.17; Ca, 2.36. Requires for $C_{88}H_{72}O_{54}Al_2Ca_2.4H_2O$; C, 48.10, H, 3.67; Al, 2.45; Ca, 3.65%. It was not found possible to repeat the Ca analysis which seems improbable from subsequent mass spectrometric work and appears to be inexplicably erroneus.

The carminic acid was found to have the following analysis; Found, C, 51.43; H, 4.56. Requires for $C_{22}H_{20}O_{13}$, C, 51.764; H, 4.31%.

Solid state ^{13}C NMR spectroscopy

Samples of carmine prepared as described were examined alongside reference samples of carminic acid and the results are discussed in Results and discussion.

Mass spectrometry

Low resolution electrospray mass spectrometry was first used and subsequently accurate mass measurements were obtained by orbitrap negative analysis and are discussed in the Results and discussion section.

Results and discussion

There are some similarities in the structure of alizarin (**3**)[4] and carmine, both involving chelation of aluminium at a carbonyl group in the anthraquinone system. The removal of this in carminic acid by sodium borohydride reduction, which from NMR examination produced CH_2 and CH(OH) groups, resulted in no complexation. The lack of involvement of the salicylate grouping was indicated by a competitive experiment between salicylic acid and alizarin in which only the latter complexed. Alizarin and carminic acid both contain 1,2-dihydroxy systems which appear to be intrinsic to complexation as shown by experiments with xanthokermsic acid (**2**) which contains

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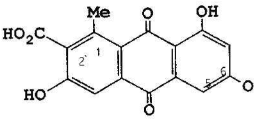


(2)

only a 5-hydroxygroup and failed to give significant complexation with alunminium.

Our preliminary experiments thus suggested that carminic acid afforded a similar structure in its calcium/aluminium complex (**3**) to that of alizarin,[4] (at that time we were not aware of the later work described).[9] For this structure, *cis/trans* isomerism appears possible and was acknowledged[4] although the *trans* form of alizarin has not been isolated and the wide separation of the two phenoxide ions from the calcium ion makes it an unlikely structure. In HPLC experimentation (G. Marshall, private communication, 1999), twin peaks have been noted, suggesting the presence of stereoisomers and LC/MS would be useful.

A possible dimeric structure for carmine in theory could involve either (**3a**) or the more hindered (**3b**), a formulation sterically less likely from the proximity of the two methyl and the glucose groups, although acceptable for the two anions adjacent to the calcium cation affording facile salt formation.

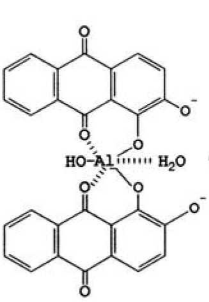
Mass spectrometry

In the course of mass spectrometry some evidence was obtained that the dimeric structure was inadequate and that carmine contained tetrameric and even polymeric materials. Originally suggestions[5,6,8] of a tetrameric structure involving three calcium and two aluminium atoms were dismissed[4] as unlikely. In MS determinations, (using electrospray) after many runs, the molecular ion was located as the dianion; Found, *m/z* 1082; Calcd for $C_{44}H_{36}O_{26}AlCa$ $(H_2O)(OH^-)$ 1082 and hence, with $z = 2$, MWt.2164. Attempts to obtain an accurate mass for the molecular ion were not successful by conventional electrospray or FAB methods. However, some time later, negative ion electrospray MS proved feasible.

Orbitrap negative ion determinations: By this procedure, double and triple charged negative ions were found with masses shown in Table 1, but none singly charged.

The results suggest that the tetramer form (molecular weight, MWt.2164) minus $2H_2O$, namely MWt.2128 is present, although no singly charged material, MWt.2127 is present. The accurate mass measurement of the double charged species suggest that $2H_2O$ and 2H have been lost. Overall, however, it is apparent that the carmine ion is heterogeneous. It is possible also that some dimer is present.

The mass range investigated was M 200–3000. It is thought that at the temperature used molecular fragmentation was low in two runs at 275 °C and at 200 °C. The accuracy of the instrument is 3 ppm and all spectra were acquired at a resolution of 100,000 at m 400.



(3)

Table 1 Masses of charged ions in negative orbital experiments

Formula	Charge state	Calculated mass	Observed mass
$C_{88}H_{71}O_{54}Al_2Ca_2$	3	708.3903	708.3892
$C_{88}H_{72}O_{54}Al_2Ca_2$	2	1063.0891	1063.0869
$C_{88}H_{73}O_{54}Al_2Ca_2$	1	127.1854	Not observed

FT IR spectroscopy

IR spectra of carmine and of carminic acid in KBr discs were compared giving the following results.

Carmine, 3412 (OH, s), 2922(w), 2849(m), 1633(m), 1568(m), 1466(m), 1409(m), 1287(m), 1079(w), 1046(w),1005(w), 777(w), 667, 614(w),541(w) cm^{-1}, (s, strong; m, medium; w, weak).

Carminic acid, 3403 (OH, s), 29 23(w), 2842(w), 1714 (m), 1615(m), 1565 (m), 1442(m), 1379(w), 1252 (s), 1225(s), 1080(m), 1039(m), 885(w), 817(w), 590(w), 459(w) cm^{-1}. Both spectra are similar and the band at 1714 cm^{-1} may be associated with the carboxyl group.

Solid state ^{13}C spectroscopy

Our results for the determination of solution spectra of carminic acid (in DMSO) agree with those found earlier.[10]

Table 2 shows a comparison of the solid state spectra of carminic acid and of the same sample of carmine as used for mass spectrometry (chemical shifts in ppm).

Formation of carmine is considered to involve the 10-carbonyl group, the 3, 5 and 6 oxygen atoms and the 2-carboxyl group. Comparison of the chemical shifts of these positions in the solid state spectra of carminic acid and of carmine show significant differences which support the proposed structural formulation.

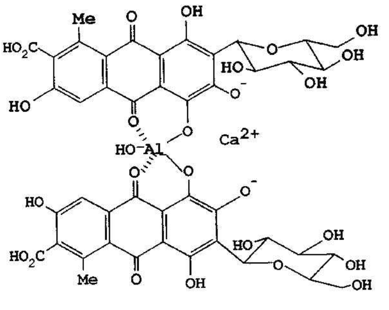
Structure of carmine

Mass spectrometry supports the view that carmine is tetrameric and that aluminium and calcium are both involved in chelation/salt formation. If the dianion is considered, a compensating Ca^{2+} would be involved and it would be necessary for the tetrameric strucure to contain aluminium and calcium with covalent and electrovalent bonds, the trivalency of aluminium and the divalency being preserved.

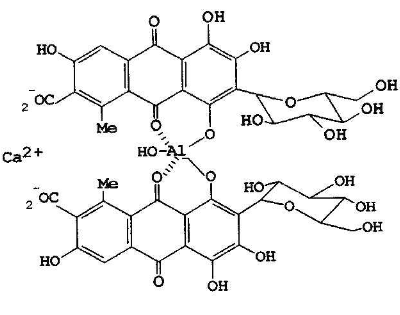
Two structures appear possible, the first (**4**, R = glucosyl) in which one calcium atom is chelated and the other is present as a cation,

Table 2 Solid state (ss) and solution (soln) spectra of carminic acid and carmine

Position assignment	Carminic acid (soln)	Carminic acid (ss)	Carmine (ss)
9,10	186.171, 186.012	183.859	184.277
CO_2H	168.030	170.163	171.599
C6	159.779	159.963	164.507
C1	157.779	157.729	156.444
C3	153.984	153.941	–
C4	147.514	147.239	147.264
C8	139.857	141.896	138.569
C4b	135.569	133.883	–
C7	132.036	130.0337	–
C8a	123.100	122.275	124.823
C2	121.240	119.798	119.966
C5	111.398	112.416	113.214
C4b	111.224	111.959	113.020
C8b	104,952	105.033	104.616

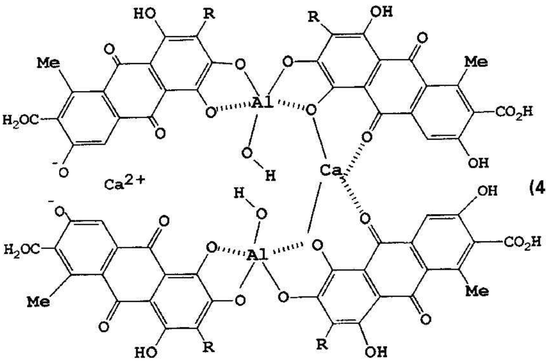


(3a)



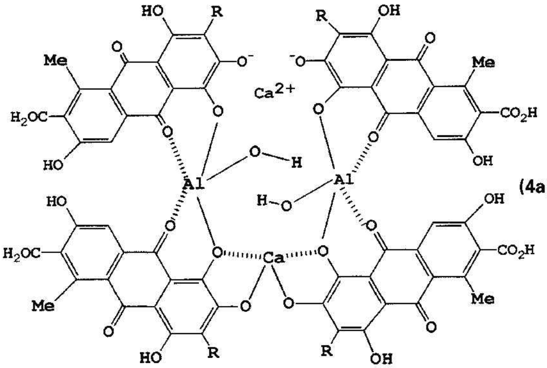
(3b)

together with two aluminium atoms present in five-membered rings, follow the proposed model[9] for alizarin.



(4)

In the second or alternative structure (**4a**), aluminium atoms are involved in chelation at the anthraquinone carbonyl group and one calcium atom is present in a five-membered ring, the other as a cation. The presence of aluminium in a five-membered ring simulates that of boron. [11]



(4a)

An inherent problem with the orbitrap negative ion method involved for **4** and **4a** is that the derived information relates to transient ions produced, although the initial substrates are essentially neutral species.

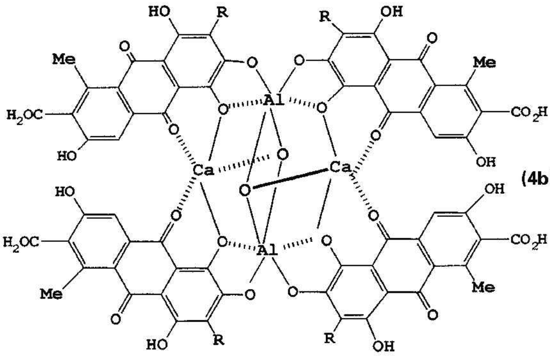
The nature of the chelating atoms could be clarified by X-ray structural analysis as with alizarin[9] but none of the samples of carmine have been found to have the required crystallinity. However, by molecular modelling with carmine on the basis of the X-ray structure for the related material alizarin,[9] a feasible neutral structure can be generated. One necessary aspect is that the carmine complex is likely to be formed from considerations[9] of that for alizarin from the reactant $Al(OAc)_3$ where reaction is depicted[9] from Al(OR), where R = OAc. Nevertheless, the intermediate involved in the case of carmine remains unclear. Two or more Al–OH bonds may undergo dehydration.

Thus, a third neutral tetrameric structure (**4b**) of carmine modelled on that[9] of alizarin would contain Al–O–Al bonds rather than two Al–OH bonds.

This posseses the correct molecular formula, $C_{88}H_{72}O_{54}Al_2Ca_2$ and appears to be the most satisfactory structural representation for carmine.

The structure **5** for alizarin based[9] on an X-ray study is shown.

One possible, but hypothetical, mode of formation of the tetrameric structure would seem to be by nucleophilic attack of phenoxide ions on aluminium and calcium ions with elimination of acetate to form a dimer, aluminium involved in a five-membered ring, and chelation of calcium with the anthraquinone system.



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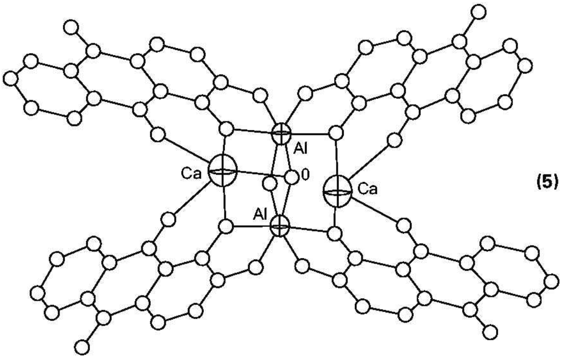

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An article entitled 'Structure of acid-stabilised carmine' does not deal with carmine but with 5-aminocarminic acid and is unfortunately an erroneus use of the term carmine which relates to metal derivatives of carminic acid.[12]

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