The Bacterial Pigment from *Pseudomonas lemmonieri*; Structure of a Degradation Product

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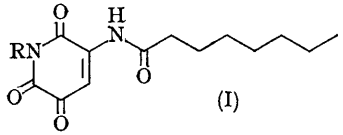
THE bacterium, *Ps. lemmonieri*, elaborates an intensely blue, highly insoluble, quinonoid-type pigment which we have not yet obtained crystalline. This metabolite contains carbon, hydrogen, nitrogen, and oxygen together with a varying amount of metallic residue (principally calcium and iron): but whether this constitutes an integral part of the pigment is not yet certain.

Oxidation of the metabolite with chromic oxide–sulphuric acid furnishes an optically inactive (o.r.d.), pale yellow product, $C_{13}H_{18}N_2O_4$, m.p. 162° having λ_{max} 208, 261, and 348 mμ (log ϵ 4·02, 4·14, and 3·80 respectively in, ethanol) now shown to have structure (I; R=H). The infrared spectrum (in KBr) shows the presence of four carbonyl groups with ν_{max} at 1742, 1690, 1664 and 1613 cm.$^{-1}$, and of two amide residues with ν_{max} at 3280 and 3320 cm.$^{-1}$ The formation of a mono-2,4-dinitrophenylhydrazone, $C_{19}H_{22}N_6O_7$, having λ_{max} 460 mμ (log ϵ 4·67, in $CHCl_3$) clearly shows that one of the carbonyl functions is a conjugated ketone. In solution (in $CHCl_3$) the carbonyl bands remain unchanged but the two NH stretching bands are replaced by a single peak at 3375 cm.$^{-1}$. Methylation of (I; R=H) furnihses an *N*-methyl derivative, $C_{13}H_{17}N_2O_4(Me)$, (I; R=Me), in which ν_{max} at 3320 cm.$^{-1}$ is absent, whilst the peak at 3375 cm.$^{-1}$ remains, in both the solid and solution spectra, in agreement with the *N*-methylation of an imide residue. Methylation of the pigment furnishes a non-crystalline *N*-methyl derivative which on oxidation with chromic oxide–sulphuric acid yields (I; R=Me), thus indicating the presence of imide functions in the pigment itself.

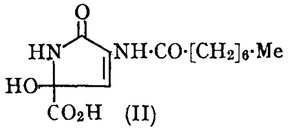
The n.m.r. spectrum of (I; R=H) [in $(CD_3)_2CO$] shows signals at τ 9·10 (triplet, $CH_2{\cdot}CH_3$: 3 protons), τ 8·66 (4 × CH_2: 8 protons), τ 8·4—7·9 (>CH_2, 2 protons), τ 7·32 (methylene adjacent to CO, 2 protons), τ 2·21 ($-\overset{|}{C}=\overset{|}{C}-H$; 1 proton), τ 0·79 (–N*H*·CO–; 1 proton) and τ – 1·03 (–CO–N*H*–CO–; 1 proton). The proton at τ – 1·03 exchanges rapidly and that at τ 0·79 less rapidly with D_2O in neutral solution. The absence of the signal at τ – 1·03 together with a signal at τ 6·6 (N–CH_3; 3 protons) in the n.m.r. spectrum of (I; R=Me) is in agreement with the conversion of an imide into an *N*-methylimido-residue.

Mass-spectral examination of (I; R=H) clearly showed a molecular ion at *m/e* 266, consistent with the formula, $C_{13}H_{18}N_2O_4$. Peaks at *m/e* 57 and *m/e* 43 corresponded to n-butyl and n-propyl residues from fission of the side-chain. Although there were no significant peaks at *m/e* values corresponding to alkyl groups higher than C_4, an accurate mass measurement upon a peak at *m/e* 127 ($C_8H_{15}O$) unequivocally established the presence of the group $CO[CH_2]_6{\cdot}Me$.

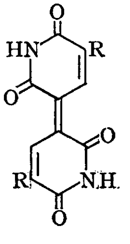
The very small quantity of (I; R=H) available did not allow us to provide a unique chemical solution to the structural problem.



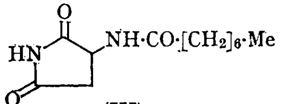
(I)



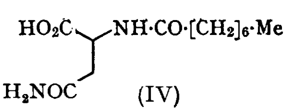
(II)



(V)



(III)

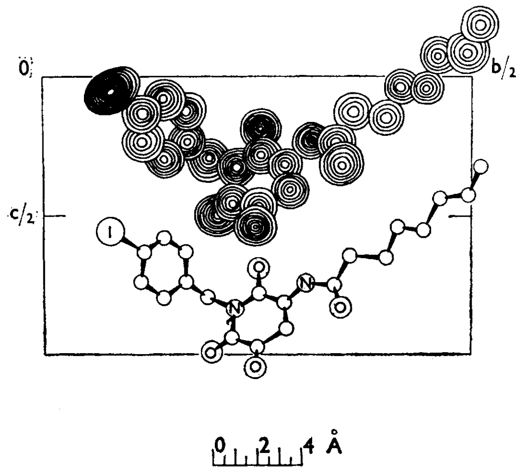


(IV)

But definition of the oxidation product as (I; R=H) has been provided by a complete three-dimensional *X*-ray analysis of the *N*-*p*-iodobenzyl derivative (I; R=*p*-iodobenzyl), the crystals of which were very thin plates not really suitable for accurate measurements. However, from equi-inclination Weissenberg photographs some 1200 independent structure amplitudes were evaluated, and this was sufficient to establish the constitution of the derivative.

The *N*-*p*-iodobenzyl derivative crystallised in the orthorhombic system, space group *Pbca* with 8 molecules of $C_{20}H_{23}N_2O_4I$ in a unit cell of dimensions a = 8·68, b = 38·71, c = 12·53 Å. The position of the iodine atom was determined from Patterson vector maps in projection in (100) and (001)[1] and confirmed by examination of the three-dimensional Harker sections. The structure was elucidated by the heavy-atom method and refined by Fourier and least-squares methods on the Glasgow University K.D.F.9 computer.[2] The value of *R* is now 13% and refinement of the atomic parameters is continuing. The conformation of the molecule in the crystal is illustrated in the Figure.

Degradation of (I; R=H) with alkali occurs rapidly, presumably by way of (II) to yield successively the imide (III) and thence (±)-n-octanoyl asparagine (IV). The compounds (III) and (IV) have been synthesised by standard methods.



FIGURE

Superimposed sections of the third three-dimensional electron-density distribution drawn parallel to **(100)**, *and the corresponding atomic arrangement.*

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[1] D. M. Hawley, B.Sc. Thesis, Glasgow, 1965.

[2] Using programmes devised by the Glasgow group; Fourier programme: J. G. Sime; least-squares programme: D. W. J. Cruickshank and J. G. F. Smith.

The compound (I; R=H) has obvious affinities with the pigments of types (V; R=OH) and (V; R=NH_2) recently isolated[3] from related microorganisms. It thus seemed possible that our pigment had the structure (V; R=$NHCO[CH_2]_6{\cdot}Me$). This was synthesised by the acylation of (V; R=NH_2)[4] with n-octanoyl chloride. Like the parent pigment (V; R=$NHCO[CH_2]_6{\cdot}Me$) could not be crystallised and although similar to the metabolite is obviously not identical with it. Oxidation of (V; R=$NHCO[CH_2]_6{\cdot}Me$) with potassium permanganate–sulphuric acid gave (I; R=H), thus furnishing a formal, total synthesis of (I; R=H) since (V; R=NH_2) was obtained by standard reactions[5] from citrazinic acid.

Examination (by g.l.c.) of the volatile acids formed from the blue pigment upon acidic hydrolysis showed the presence of only n-octanoic acid. Further investigations are in progress.

All compounds had the requisite spectral and analytical characteristics.

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[3] R. Kuhn, H. Bauer, H.-J. Knackmuss, D. A. Kuhn, and M. P. Starr, *Naturwiss.*, 1964, **51**, 409.

[4] R. Kuhn, H. Bauer, H.-J. Knackmuss, *Chem. Ber.*, 1965, **98**, 2139.

[5] W. J. Sell and T. H. Easterfield, *J. Chem. Soc.*, 1893, **63**, 1035; W. J. Sell and H. Jackson, *ibid.*, 1899, **75**, 507.