The Bacterial Pigment from *Pseudomonas lemonnieri*. Part 1. Structure of a Degradation Product, 3-n-Octanamidopyridine-2,5,6-trione, by *X*-Ray Crystallography

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Degradation with chromic acid of the blue pigment, lemonnierin, $C_{26}H_{37}N_5O_6$, from *Pseudomonas lemonnieri*, gives 3-n-octanamidopyridine-2,5,6-trione (1 ; R = H), the structure of which has been defined by *X*-ray analysis of the *N*-*p*-iodobenzyl derivative (1 ; R = *p*-iodobenzyl). Crystals are orthorhombic, space group *Pbca* with eight molecules of $C_{20}H_{23}IN_2O_4$ in a unit cell of dimensions $a = 8.68(3)$, $b = 38.71(5)$, $c = 12.53(3)$ Å. Intensity data were obtained by visual estimation of Weissenberg films. The structure was solved by the heavy-atom method and refined by block-diagonal least-squares calculations : $R = 0.122$ for 1 004 measured reflections.

Degradation of (1 ; R = H) with alkali gives successively (±)-n-octanamidosuccinimide (2) and (±)-n-octanoyl-asparagine (4), which have been synthesised.

The formal synthesis of (1 ; R = H) is recorded.

THE bacterium, *Ps. lemonnieri*, elaborates an intensely blue pigment which we have not been able to crystallise. The pigment, which we call lemonnierin, contains carbon, hydrogen, nitrogen, and oxygen together with varying amounts of associated inorganic ions (principally calcium and iron) which may be removed by solution in acetic acid. We do not believe that these ions constitute an integral part of the pigment, and will discuss this aspect in a later publication. The insolubility of lemonnierin in the majority of solvents, combined with its non-crystallinity made the normal criteria of homogeniety unhelpful. Even the n.m.r. spectrum (in CF_3CO_2H) exhibited only an envelope of signals, τ 9.06—7.01 (m), arising from the n-heptyl residue (see later); more diagnostically useful signals were absent. However, although lemonnierin decomposes slowly on t.l.c., and forms rather diffuse spots with 'tailing', this technique seemed to indicate the essential homogeneity of our pigment: further work (to be reported later) confirms this view. Mass spectrometry indicated a molecular formula, $C_{26}H_{37}N_5O_6$, in agreement with numerous elemental analyses. The i.r. spectrum (KBr) showed *inter alia* $\nu_{max.}$ at 3 260m, 3 200m, 1 675s, 1 655s, 1 645s, 1 640s, 1 636m, and 1 500s cm^{-1}. The u.v. spectrum exhibited a single absorption band at λ 625 nm (log ε 4.75) in pyridine, acetic acid or dimethylformamide. This paper records the structure definition of a major degradation product of lemonnierin.

A variety of reagents was explored before we found that oxidation of the pigment on a small scale, with Jones' reagent furnished an optically inactive (o.r.d.) pale yellow, crystalline product, $C_{13}H_{18}N_2O_4$. The i.r. spectrum (KBr) showed the presence of four carbonyl groups with $\nu_{max.}$ 1 742, 1 690, 1 664, and 1 613 cm^{-1}, and of two amide residues with $\nu_{max.}$ 3 280 and 3 320 cm^{-1}.

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The formation of a mono-2,4-dinitrophenylhydrazone, $C_{19}H_{22}N_6O_7$, with a $\lambda_{max.}$ 460 nm (log 4.6) in $CHCl_3$, showed that one of the carbonyl residues is part of a conjugated system. Methylation of (1; R = H) furnished an *N*-methyl derivative, $C_{13}H_{17}N_2O_4(Me)$ (1; R = Me), devoid of the i.r. peak at $\nu_{max.}$ 3 320 cm^{-1}, in agreement with the *N*-methylation of an imide residue. Methylation of lemonnierin furnished a non-crystalline *N*-methyl derivative which on oxidation with Jones' reagent yielded (1; R = Me), thereby indicating the presence of one or more imide functions in the pigment.

Because of the insolubility of (1; R = H) in chloroform the n.m.r. spectrum was determined in $(CD_3)_2CO$ and showed signals at τ 9.10 (t, CH_2CH_3, 3 H), 8.66 (m, 4 × CH_2, 8 H), 8.4—7.9 (m, CH_2, 2 H), 7.32 (m, $COCH_2$, 2 H), 2.21 (s, C=C-*H*, 1 H), 0.79 (s, N*H*·CO-, 1 H), and −1.03 (s, CON*H*·CO, 1 H). The proton at τ −1.03 exchanged rapidly and that at τ 0.79 less rapidly with deuterium oxide in neutral solution. The

(1)
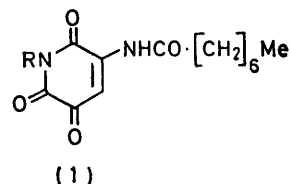
absence of the signal at τ −1.03 in the spectrum of (1; R = Me) is in agreement with the methylation of an imide (10; R = H) to a methyl imide (1; R = Me).

The mass spectrum of (1; R = H) showed a strong molecular ion at *m/e* 266, consistent with the formula $C_{13}H_{18}N_2O_4$, whilst peaks at *m/e* 57 and 43 corresponded to n-butyl and n-propyl fragments respectively; an accurate mass measurement upon a peak at *m/e* 127 ($C_8H_{15}O$) established the presence of the group CO-$[CH_2]_6$·CH_3.

The very small quantity of (1; R = H) initially

available, combined with its relative inaccessibility, did not permit a unique structural solution from the evidence already discussed.

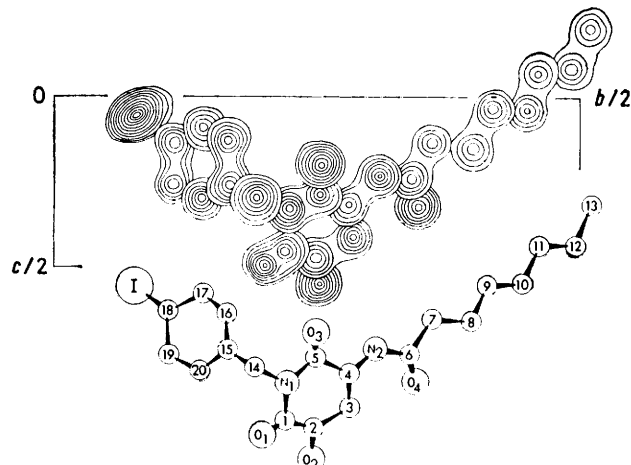We therefore explored the preparation of a suitable

FIGURE 1 View of the molecule (1; R = p-IC$_6$H$_4$CH$_2$) illustrating the crystallographic numbering scheme

derivative for X-ray crystallography, and after the formation of several N-alkylated derivatives were ultimately able to provide [1] a unique structural definition of the derivative (1; R = p-iodobenzyl), the structure of which and the numbering scheme adopted for the analysis are in Figure 1. The heterocyclic ring is planar within

contribution to the structure from the corresponding enol. The remaining bond distances are in accord with expected values [2] except the C(4)–N(2) (1.33 Å) and C(6)–N(2) (1.36 Å) bonds which are shorter than the single bond distance of 1.47 Å and indicate a degree of conjugation in the region involving the double bonds C(3)–C(4) and C(6)–O(4). The angle between the planes specified by N(2), O(4), C(6), C(7) and N(2), C(2), C(3), C(4), C(5), is only 7.7° which would not interfere unduly with conjugation effects. This small interplanar angle results in a close intramolecular contact [C(3) · · · O(4) (2.81 Å)]. The n-C$_7$H$_{15}$ side chain adopts a fully extended staggered conformation such as is commonly observed in crystal structures of long-chain fatty acids.[3]

In the crystal structure (Figure 2) molecules have numerous contacts <3.5 Å in the a- and c-directions. The N–H group at N(2) makes two short contacts with neighbouring carbonyl groups, but as hydrogen atoms were not located, no hydrogen-bond assignments were made. There are no contacts <3.5 Å between the ends of alkyl chains of neighbouring molecules. The difficulty we experienced in obtaining suitable crystals is thus easily understood. The crystals grew satisfactorily in the a- and c-directions (where hydrogen bonding and van der Waals contacts occur) but indifferently in the b-direction (where there are no short intermolecular contacts); very thin plates with the [010] face well developed were thus obtained.

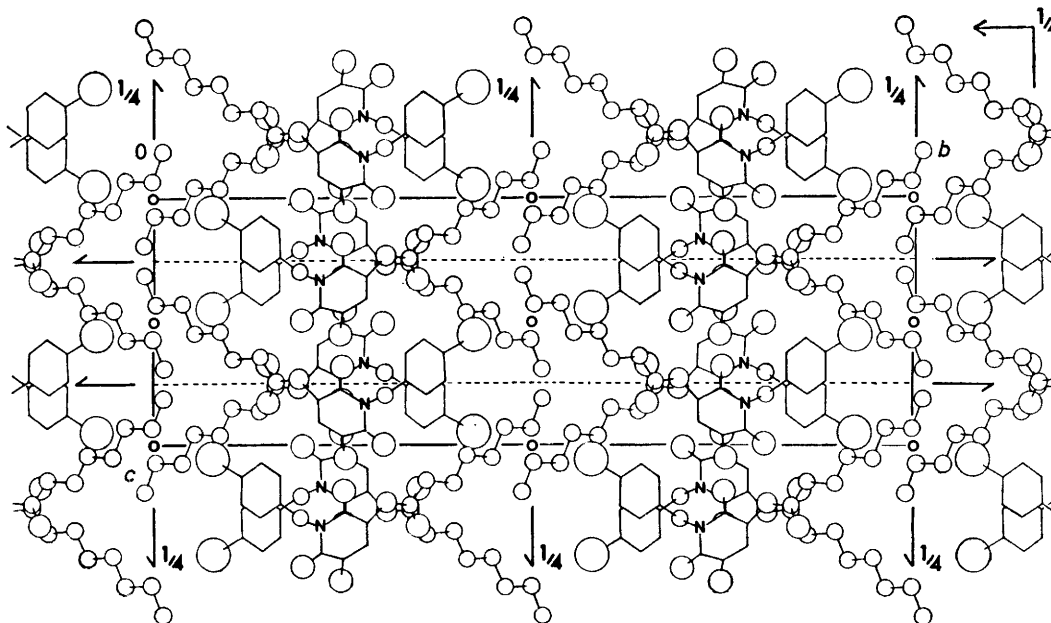At this stage, and with further, albeit small, quantities

FIGURE 2 The crystal structure of (1; R = p-IC$_6$H$_4$CH$_2$) as seen in its a-axis projection

the accuracy of the analysis (maximum deviations from the ring plane are ±0.05 Å). Bond distances (Table 2) are consistent with the heterocyclic ring being formulated as a triketo (imido) derivative; the C=O bonds (mean 1.24 Å) and C–N bonds (mean 1.44 Å) indicate no

of (1; R = H) available it was possible to investigate the chemistry of the oxidation product. Degradation occurred rapidly in the presence of sodium carbonate solution to yield the (±)-succinimide (2), presumably by a benzilic acid-type rearrangement to yield (3), which

rapidly decarboxylated to form the imide; (2) was synthesised as follows. Asparagine was acylated with n-octanoyl chloride to yield the amide (4), which formed the ($\pm$)-imide (2) upon heating. Hydrolysis of the natural imide (2) with sodium hydroxide solution gave ($\pm$)-n-octanoylasparagine (4). The synthesis of some associated derivatives is described in the Experimental section.

A formal synthesis of (1; R = H) was achieved as follows. Acylation of 5,5'-diamino-3,3'-dihydroxy-3,3'-bipyridylidene-2,2'quinone [4] (5; R = H), with n-octanoyl chloride gave the amide (5; R = CO[CH$_2$]$_6$·Me).

(2)

(3)

(4)

(5)
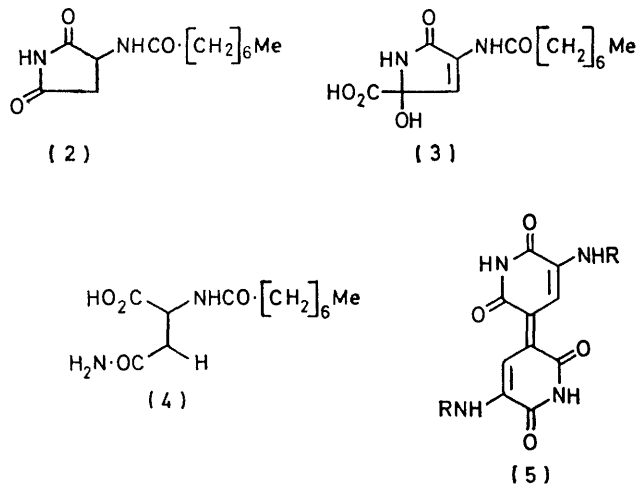
Oxidation of this with acidic aqueous potassium permanganate solution gave (1; R = H) identical with the product from lemonnierin. The mass spectrum of (5; R = CO[CH$_2$]$_2$Me) showed strong peaks at m/e 374 (i.e. $M - 126$), and m/e 248 (i.e. $M - 252$) corresponding to the loss of one and two octanoyl residues respectively.

Our specimen of lemonnierin apparently contained only n-octanoyl residues, devoid of congeners. Thus lemonnierin was hydrolysed with sulphuric acid; the acidic fraction was extracted with methylene chloride, and the extract methylated with diazomethane. Analysis by g.l.c. showed the presence of only methyl n-octanoate by comparison with reference samples of the methyl esters of acetic, propionic, n-hexanoic, and n-octanoic acids.

In 1967, two years after our initial report,[1] Knackmuss et al.[4,5] claimed, with minimal supporting evidence and with no reference to our prior work,[1] the isolation of the 2,5,6-trione (1; R = H) from oxidation with nitric acid of a specimen of lemonnierin.

The identity (or otherwise) of these authors' blue pigment with ours, together with our structural conclusions, will be discussed in a later paper.

EXPERIMENTAL

Growth of Ps. lemonnieri.—The organism was grown upon a medium containing Difco yeast extract (1%), glucose (2%), and calcium carbonate (2%), made up with tap water. No pigmentation occurred using the same medium in *distilled*

water: it was thus inferred that the trace elements in tap water are necessary for pigmentation. Similar observations have been made for other *Ps.* species.[4] The mixture was sterilised at 15 lb in^{-2} of steam pressure during $\frac{1}{2}$ h, and spread upon trays in thin layers to solidify. After solidification the medium was treated with an inoculum of *Ps. lemonnieri*, and incubated at 25 °C for 3 weeks. The resultant blue bacterial mass was scraped from the surface of the medium, and slurried with water. The cell mass was separated by centrifuging, washed with methanol containing 0.5% hydrochloric acid, and dried to yield a blue, free running powder. This was ground in a ball-mill and a mixture of the resultant powder (50 g) and white sand (200 g) was extracted with dried pyridine in a Soxhlet during 2 h, with a cycle period of *ca.* 10 min. The hot solution was immediately evaporated *in vacuo* at 40 °C (nitrogen) to 50 ml, and diluted with acetone (200 ml). The blue precipitate was separated (centrifuge) and washed three times with acetone (centrifuge) and dried to yield lemonnierin as a non-crystalline blue powder (0.1 g), m.p. 300 °C (decomp.). Extensive attempts to crystallise lemonnierin failed.

Increased extraction time gave a decreased yield as did allowing the pyridine extract to stand overnight before evaporation, although solutions in pyridine of purified lemonnierin were stable for 2—3 weeks.

The pigment thus extracted was soluble in pyridine and trifluoroacetic acid but insoluble in most common solvents.

Solution in boiling acetic acid followed by reprecipitation on cooling (3 times) gave an almost ash-free product (Found: C, 60.1, 59.9; H, 7.2, 7.1; N, 12.9, 12.3; ash 0.2, 0.2%; M^+ 515.274 9. $C_{26}H_{37}N_5O_6$ requires C, 60.6; H, 7.2; N, 13.6%; M 515.274 4).

Oxidation of Lemonnierin.—A solution of Jones' reagent was added dropwise to a stirred mixture of lemonnierin (0.05 g) and water (0.5 ml) until the blue colour was discharged. After addition of water (10 ml) and isolation with ether, 3-*n-octanamidopyridine*-2,5,6-*trione* (0.01 g) formed pale yellow plates, m.p. 161 °C from acetone–light petroleum (b.p. 60—80 °C) (1 : 1) or from benzene (Found: C, 58.3; H, 6.8; N, 10.6. $C_{13}H_{18}N_2O_4$ requires C, 58.6; H, 6.8; N, 10.5%), $\lambda_{max.}$ 220 (log ε 4.02), 263 (4.14), and 349 nm (3.80) (in ethanol).

Larger quantities of this product were obtained by performing more oxidations—attempts to increase the scale of the oxidations were not successful.

The 2,4-*dinitrophenylhydrazone* formed crimson microprisms, m.p. 259 °C from benzene (Found: C, 50.7; H, 5.1; N, 18.6. $C_{19}H_{22}N_6O_7$ requires C, 51.1; H, 5.0; N, 18.8%), $\lambda_{max.}$ 460 nm (log ε 4.65) (in chloroform).

Methylation of 3-n-octanamidopyridine-2,5,6-trione (0.15 g) in boiling acetone (10 ml) containing sodium hydrogen carbonate (1 g) and methyl iodide (1 ml) during 3 h, gave the N-*methyl derivative* (0.1 g) as yellow plates, m.p. 136 °C from benzene–ether (Found: C, 59.5; H, 7.3; N, 10.0. $C_{14}H_{20}N_2O_4$ requires C, 60.0; H, 7.2; N, 10.0%).

Prepared similarly but using benzyl iodide, the N-*benzyl derivative* formed yellow plates (quantitatively), m.p. 117 °C from ether–light petroleum (b.p. 40—60 °C) (Found: C, 67.6; H, 6.6; N, 8.0. $C_{20}H_{24}N_2O_4$ requires C, 67.4; H, 6.8; N, 7.9%).

The N-m-*iodobenzyl derivative* formed yellow prisms, m.p. 117 °C (from ether) (Found: C, 49.1; H, 4.9; I, 26.7; N, 5.6. $C_{20}H_{23}IN_2O_4$ requires C, 49.8; H, 4.8; I, 26.3; N, 5.8%).

The N-p-*iodobenzyl derivative* formed yellow plates, m.p.

174 °C (from acetone) (Found: C, 48.6; H, 5.0; I, 25.6; N, 5.9%).

X-*Ray Crystal Structure Analysis of* N-p-*Iodobenzyl*-3-n-*octanamidopyridine*-2,5,6-*trione* (1; R = p-IC$_6$H$_4$CH$_2$).— The crystals were in the form of fragile thin plates which resulted in X-ray photographs of very poor quality. Cell

TABLE 1

Final fractional co-ordinates ($\times 10^4$) for (1; R = p-IC$_6$H$_4$CH$_2$) and isotropic thermal parameters ($\times 10^3$). Estimated standard deviations are in parentheses

Atom	x/a	y/b	z/c	U_{iso}
I	1 007(3)	772(1)	538(3)	*
O(1)	−1 456(19)	1 956(4)	4 941(15)	55(5)
O(2)	503(24)	2 468(5)	5 484(16)	80(6)
O(3)	−2 330(20)	2 508(5)	1 834(15)	61(5)
O(4)	586(26)	3 481(6)	3 266(19)	99(7)
N(1)	−1 857(22)	2 209(5)	3 329(16)	44(5)
N(2)	−751(22)	3 067(5)	2 349(17)	47(6)
C(1)	−1 151(31)	2 209(7)	4 374(21)	55(7)
C(2)	−226(32)	2 515(7)	4 654(21)	57(8)
C(3)	−199(29)	2 827(6)	4 063(20)	45(7)
C(4)	−875(27)	2 822(6)	3 081(19)	43(7)
C(5)	−1 719(28)	2 524(6)	2 733(20)	44(7)
C(6)	−46(34)	3 380(7)	2 435(26)	68(8)
C(7)	−67(41)	3 592(8)	1 426(26)	87(10)
C(8)	758(35)	3 951(8)	1 521(26)	77(9)
C(9)	776(33)	4 120(7)	397(23)	66(9)
C(10)	1 522(33)	4 459(8)	487(23)	68(9)
C(11)	1 570(45)	4 622(10)	−722(31)	108(13)
C(12)	2 418(41)	4 976(9)	−683(31)	97(11)
C(13)	2 519(63)	5 121(13)	−1 876(46)	177(22)
C(14)	−2 796(27)	1 930(6)	2 978(19)	42(7)
C(15)	−1 917(28)	1 665(6)	2 394(21)	44(7)
C(16)	−1 856(32)	1 661(7)	1 281(22)	56(8)
C(17)	−995(32)	1 418(8)	731(23)	67(8)
C(18)	−280(34)	1 168(7)	1 264(24)	67(9)
C(19)	−374(37)	1 148(8)	2 422(30)	82(10)
C(20)	−1 135(34)	1 398(8)	2 968(24)	73(9)

* The thermal vibration of the iodine atom is represented by the following equation: $\exp[-2\pi^2(0.101\ h^2a^{*2} + 0.093\ k^2b^{*2} + 0.154\ l^2c^{*2} - 0.111\ klb^*c^* + 0.052\ lhc^*a^* - 0.002\ hka^*b^*)]$.

dimensions [6] and space group were determined from rotation and Weissenberg photographs taken about the a and c axes with Cu-K_α radiation.

Crystal Data.—C$_{20}$H$_{23}$IN$_2$O$_4$, M = 482.3, orthorhombic, a = 8.68(3), b = 38.71(5), c = 12.53(3) Å, U = 4 210 Å^3, D_m = 1.51, Z = 8, D_c = 1.52 g cm^{-3}, $F(000)$ = 1 936, 20 °C, Cu-K_α radiation, λ = 1.542 Å, μ = 126 cm^{-1}. Space group *Pbca* determined uniquely from systematic absences $0kl$ when $k = 2n + 1$, $h0l$ when $l = 2n + 1$, $hk0$ when $h = 2n + 1$.

Intensity data were obtained by visual estimation of multiple-film Weissenberg photographs of the $0kl$—$7kl$, and $hk0$ reciprocal lattice nets. The Weissenberg films were of poor quality (mainly because even the ' best ' ' single ' crystal was really an accretion of many very thin plates) but these films yielded sufficient data to allow the structure to be defined. Rapid fall-off of the data with $\sin \theta / \lambda$ resulted in a relatively small number of observed reflections (21% of the total theoretically accessible to Cu-K_α radiation). Lorentz, polarisation, and rotation factors were applied to the X-ray intensities and the resulting 1 004 measured structure amplitudes were put on a common scale by comparison with reflections from the $hk0$ zone. The overall scale factor was initially determined by ensuring that $k\Sigma|F_o| = \Sigma|F_o|$ and ultimately by least-squares methods.

Structure Analysis.—The structure was solved by application of the heavy-atom method. The position of the iodine

atom was obtained from a three-dimensional Patterson function and two rounds of electron-density and structure factor calculations revealed the entire structure. Refinement was by block-diagonal least-squares calculations using a weighting scheme $w = [1/(A + F_o + B|F_o|^2)]^{\frac{1}{2}}$ where $A = 2\ F_{min.}$ and $B = 2/F_{max.}$. No allowance was made for hydrogen atoms in any of the calculations. The iodine atom was allowed anisotropic thermal parameters, but all carbon, nitrogen, and oxygen atoms were constrained to isotropic thermal vibration. The refinement converged when $R = 0.122$. A final (F_o-F_c) Fourier synthesis showed no errors in the structural model and gave a fluctuating background in the region of ± 0.3 e Å^{-3}

TABLE 2

Interatomic distances and angles in (1; R = p-IC$_6$H$_4$CH$_2$) with estimated standard deviations in parentheses

(a) Bond lengths (Å)

I–C(18)	2.10(3)	C(6)–C(7)	1.51(5)
O(1)–C(1)	1.24(3)	C(7)–C(8)	1.57(5)
O(2)–C(2)	1.23(3)	C(8)–C(9)	1.55(4)
O(3)–C(5)	1.25(3)	C(9)–C(10)	1.47(4)
O(4)–C(6)	1.24(3)	C(10)–C(11)	1.64(5)
N(1)–C(1)	1.45(3)	C(11)–C(12)	1.56(5)
N(1)–C(5)	1.44(3)	C(12)–C(13)	1.60(7)
N(1)–C(14)	1.42(3)	C(14)–C(15)	1.47(3)
N(2)–C(4)	1.33(3)	C(15)–C(16)	1.40(4)
N(2)–C(6)	1.36(3)	C(15)–C(20)	1.43(4)
C(1)–C(2)	1.47(4)	C(16)–C(17)	1.38(4)
C(2)–C(3)	1.42(4)	C(17)–C(18)	1.33(4)
C(3)–C(4)	1.36(4)	C(18)–C(19)	1.46(5)
C(4)–C(5)	1.43(3)	C(19)–C(20)	1.36(4)

(b) Bond angles (mean standard deviation is 2°)

C(1)–N(1)–C(5)	116	N(2)–C(6)–C(7)	114
C(1)–N(1)–C(14)	122	C(6)–C(7)–C(8)	114
C(5)–N(1)–C(14)	122	C(7)–C(8)–C(9)	108
C(4)–N(2)–C(6)	128	C(8)–C(9)–C(10)	108
O(1)–C(1)–N(1)	115	C(9)–C(10)–C(11)	107
O(1)–C(1)–C(2)	128	C(10)–C(11)–C(12)	109
N(1)–C(1)–C(2)	117	C(11)–C(12)–C(13)	108
O(2)–C(2)–C(1)	111	N(1)–C(14)–C(15)	113
O(2)–C(2)–C(3)	124	C(14)–C(15)–C(16)	122
C(1)–C(2)–C(3)	125	C(14)–C(15)–C(20)	120
C(2)–C(3)–C(4)	117	C(16)–C(15)–C(20)	119
N(2)–C(4)–C(3)	125	C(15)–C(16)–C(17)	122
N(2)–C(4)–C(5)	114	C(16)–C(17)–C(18)	120
C(3)–C(4)–C(5)	120	I(1)–C(18)–C(17)	124
O(3)–C(5)–N(1)	113	I(1)–C(18)–C(19)	115
O(3)–C(5)–C(4)	122	C(17)–C(18)–C(19)	121
N(1)–C(5)–C(4)	125	C(18)–C(19)–C(20)	120
O(4)–C(6)–N(2)	123	C(15)–C(20)–C(19)	120
O(4)–C(6)–C(7)	122		

(c) Intermolecular contacts $\leqslant 3.5$ Å

O(2) · · · O(3^i)	2.99	O(3) · · · C(2^{iv})	3.29
O(3) · · · C(3^{ii})	3.00	O(2) · · · N(2^i)	3.31
O(2) · · · N(1^{iii})	3.01	O(3) · · · C(4^{ii})	3.31
O(1) · · · C(7^i)	3.07	O(2) · · · C(14^{iii})	3.37
O(1) · · · N(2^i)	3.08	O(4) · · · C(17^i)	3.40
O(3) · · · C(2^{ii})	3.13	O(2) · · · C(5^i)	3.42
O(2) · · · C(1^{iii})	3.17	O(3) · · · C(1^{iv})	3.43
O(1) · · · O(3^i)	3.24	O(2) · · · O(3^v)	3.46
O(2) · · · C(5^{iii})	3.29	O(1) · · · O(2^{vi})	3.50

The Roman numerals refer to the following transformations applied to the co-ordinates of Table 1.

i	$x, \frac{1}{2} - y, \frac{1}{2} + z$	iv $x, \frac{1}{2} - y, -\frac{1}{2} + z$
ii	$-\frac{1}{2} + x, y, \frac{1}{2} - z$	v $\frac{1}{2} + x, y, \frac{1}{2} - z$
iii	$\frac{1}{2} + x, \frac{1}{2} - y, 1 - z$	vi $-\frac{1}{2} + x, \frac{1}{2} - y, 1 - z$

$[\sigma(\rho)$ was 0.22 e Å$^{-3}]$. Form-factors used were those of Freeman [7] for carbon, Berghuis *et al.*,[8] for nitrogen and oxygen, and Thomas and Umeda [9] for iodine. Atomic fractional and thermal parameters are given in Table 1. Interatomic distances and angles are in Table 2. The

N-*Methyl-lemonnierin.*—(a) A mixture of finely divided femonnierin (0.2 g), acetone (200 ml), methyl iodide (1 ml), and potassium carbonate (3 g) was refluxed during 20 h. The product was isolated in the normal way, and the solution evaporated to (5 ml) and diluted with methylene chloride (50 ml). The resultant solution was washed with water, dried, and evaporated to yield N-*methyl-lemonnierin* as a non-crystalline, deep blue powder, m.p. 195—196 °C (0.08 g) (Found: C, 62.0; H, 7.7; N, 12.7%; M^+ 543. $C_{28}H_{41}N_5O_6$ requires C, 61.9; H, 7.6; N, 12.9%; M 543), $\lambda_{max.}$ 645 nm (log ε 4.73) in methylene chloride and 635 nm (log ε 4.80) in dimethylformamide; τ (CDCl$_3$) 6.6 (s, $2 \times {>}$N·CH$_3$, 6 H). This N-methyl-lemonnierin is sparingly soluble in acetone and chloroform, and more soluble in dimethyl sulphoxide, dimethylformamide, and acetic acid.

(b) Lemonnierin (0.2 g), dimethyl sulphate (0.1 g), potassium carbonate (3 g), and diglyme (20 ml) were refluxed for 5 min, to yield after isolation as in (a) N-methyl-lemonnierin (0.15 g), m.p. 195—196 °C.

Oxidation of a solution of N-methyl-lemonnierin (0.1 g) in acetone (200 ml) with dropwise addition of Jones' reagent until the colour had been discharged gave 1-methyl-3-octanamidopyridine-2,5,6-trione (0.025 g) identical (m.p., mixed m.p., i.r., u.v., and n.m.r.) with the methylation product from (1; R = H).

Degradation of 3-n-*Octanamidopyridine-2,5,6-trione.*—(a) A solution of this trione (0.1 g) in acetone (5 ml) and ether (25 ml) was shaken with a 5% solution (10 ml) of sodium carbonate during which operation the initially yellow colour of the organic layer was discharged. The aqueous solution was separated, acidified (pH 3), and extracted with ether to yield the *imide* of (±)-N-*n-octanoylaspartic acid* (0.05 g) as prisms, m.p. 126 °C from ether (Found: C, 59.5; H, 8.2; N, 11.5. $C_{12}H_{20}N_2O_3$ requires C, 60.0; H, 8.4; N, 11.7%), identical with an authentic specimen.

(b) A solution of this imide (0.1 g) in acetone (5 ml) and ether (15 ml) was shaken with a 2% solution (4 × 10 ml) of sodium hydroxide. The combined extracts were acidified with hydrochloric acid to yield a precipitate which was purified from acetone to yield (±)-N-*n-octanoylasparagine* (0.07 g) as prisms, m.p. 150 °C (Found: C, 55.7; H, 8.5; N, 10.7. $C_{12}H_{22}N_2O_4$ requires C, 55.8; H, 8.6; N, 10.9%), identical with an authentic specimen.

Derivatives of Aspartic Acid.—(a) A mixture of (±)-asparagine monohydrate (2 g), n-octanoyl chloride (2 ml), 20% sodium hydroxide solution (4 ml), and water (10 ml) was shaken for 0.5 h. The resultant solution was acidified with hydrochloric acid and the precipitate purified from acetone to yield (±)-N-n-octanoylasparagine (2.5 g) as small prisms, m.p. 150 °C (Found: C, 55.3; H, 8.5; N, 11.0. Calc. for $C_{12}H_{22}N_2O_4$: C, 55.8; H, 8.6; N, 10.9%).

(b) When this amide (1 g) was heated at 180 °C for 3 min, the imide (0.2 g) formed prisms, m.p. 126 °C from ether, identical with that obtained from the degradation of 3-n-octanamidopyridine-2,5,6-trione.

Methylation of this imide (0.6 g) with 5% sodium carbonate solution (15 ml) and dimethyl sulphate (1.5 ml) during 1.5 h, gave the N-*methyl derivative* (0.4 g) as needles, m.p. 81° from ether (Found: C, 61.0; H, 8.5; N, 11.0. $C_{13}H_{22}N_2O_3$ requires C, 61.3; H, 8.7; N, 11.0%).

The corresponding N-*benzyl imide* formed needles, m.p. 88 °C from ether–light petroleum (Found: C, 69.0; H, 7.8; N, 8.6. $C_{19}H_{26}N_2O_3$ requires C, 69.1; H, 7.9; N, 8.5%).

(c) The *methyl ester* of (±)-N-n-octanoylasparagine (formed using diazomethane) separated from acetone as prisms, m.p. 115 °C (Found: C, 57.3; H, 8.8; N, 10.3; OMe, 11.3. $C_{12}H_{21}N_2O_3$·OMe requires C, 57.3; H, 8.9; N, 10.3; OMe, 11.4%).

(d) A solution of (±)-aspartic acid (6.7 g), n-octanoyl chloride (8 ml), and sodium hydroxide (7.2 g) in water (20 ml) was shaken for 0.5 h. After acidification of the reaction mixture to pH 2, the resultant precipitate was purified from ether to yield (±)-*n-octanoylaspartic acid* (7 g) as micro-prisms, m.p. 107—109 °C (Found: C, 55.8; H, 8.3; N, 5.4. $C_{12}H_{21}NO_5$ requires C, 55.6; H, 8.2; N, 5.4%).

(e) An ethereal solution in diazomethane (1 equiv.) was added to a solution of (±)-N-n-octanoylaspartic acid (2.6 g) in methanol (15 ml) and ether (55 ml) to yield the α-methyl ester of (±)-N-n-octanoylaspartic acid as a viscous oil, which was treated with aqueous ammonia (d 0.88; 30 ml) during 24 h, to yield the α-*monoamide* of (±)-N-n-octanoyl-aspartic acid (0.8 g) as prisms, m.p. 155 °C from acetone (Found: C, 56.4; H, 8.7; N, 10.8. $C_{12}H_{22}N_2O_4$ requires C, 55.8; H, 8.6; N, 10.9%). The structure of this amide (and hence that of the parent ester) follows from its non-identity with the isomeric (±)-N-n-octanoylasparagine.

(f) Treatment of (±)-N-n-octanoylaspartic acid (1.5 g) with an excess of ethereal diazomethane gave the (±)-*dimethyl ester* (quantitatively) as an oil, b.p. 150 °C at 0.1 mmHg (Found: C, 58.2; H, 9.0; N, 4.9. $C_{14}H_{25}NO_5$ requires C, 58.5; H, 8.8; N, 4.9%).

Synthesis of 3-n-*Octanamidopyridine-2,5,6-trione.*—A solution of 5,5'-diamino-6,6'-dihydroxy-3,3'-bipyridylidene-2,2'-quinone[5] (0.125 g), n-octanoyl chloride (0.25 ml), and pyridine (5 ml) was refluxed during 3 h to yield 5,5'-*di-n-octanamido-6,6'-dihydroxy-3,3'-bipyridylidene-2,2'-quinone* (0.13 g) as a purple powder, m.p. >300 °C from acetone (Found: C, 63.5; H, 7.0; N, 11.0%; M^+ 500.263 4. $C_{26}H_{36}N_4O_6$ requires C, 62.4; H, 7.3; N, 11.3%; M 500.263 5); τ 9.07 (3 H), 8.60 (8 H), 8.10 (2 H), 7.20 (2 H), and −0.3 (s, C-2 proton, H) (in CF$_3$CO$_2$D); $\lambda_{max.}$ 520 nm (log ε 4.39) in pyridine; $\lambda_{max.}$ 520 nm (log ε 4.39) in methylene chloride; $\lambda_{max.}$ 510 nm (log ε 4.39) in acetic acid (cf. ref. 5).

Oxidation of a solution of this quinone (0.1 g) in acetone (300 ml) with a solution of potassium permanganate [0.5% (w/v) containing 2% sulphuric acid] until the pink colour persisted for $\frac{1}{2}$ min gave, after purification by t.l.c. on silica from ether, 3-n-octanamidopyridine-2,3,6-trione (10 mg) as plates from acetone, m.p. 161 °C, identical with the product from lemonnierin (Found: C, 58.4; H, 7.0; N, 10.4. Calc. for $C_{13}H_{18}N_2O_4$: C, 58.6; H, 6.8; N, 10.5%).

Determination of the Composition of the Side-chain in Lemonnierin.—A mixture of lemonnierin (0.05 g) and 70% (w/w) sulphuric acid (5 ml) was refluxed for 2 h, by which time the resultant solution was colourless. The cooled mixture was diluted with water and extracted (5 × 10 ml) with methylene chloride, and the extract methylated with an excess of diazomethane in the same solvent. The solution was cautiously evaporated to dryness and dried by further azeotropic distillation with additional methylene chloride as necessary. The resultant product was made up to 1 ml in methylene chloride and 10-μl aliquots injected onto a 2-m column using Apiezon L (200 ml min⁻¹ N₂).

An authentic comparative sample was prepared from n-octanoic acid, the homogeneity of which was confirmed by

(*a*) g.l.c. of the methyl ester, (*b*) the m.p. of the amide, and (*c*) the t.l.c. of the amide.

Comparison of the natural sample with this authentic sample and also with a mixture of equal proportions of acetic, butyric, and n-hexanoic esters, confirmed the presence of only n-octanoic acid in the acid from natural lemonnierin.

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