798. *The Structure of Pulcherrimin.*

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New analytical and degradative evidence indicates that pulcherriminic acid [1, 2] is to be formulated as 2 : 5-di*iso*butyl-3 : 4-dihydro-4 : 6-dihydroxy-3-oxopyrazine 1-oxide (VI). Consequently pulcherrimin, a natural pigment, is to be regarded as the corresponding ferric complex (V).

The synthesis of 2 : 5-di*iso*butyl-1 : 4-dihydroxy-3 : 6-dioxopiperazine [2] (II) hitherto regarded as representing pulcherriminic acid [1] showed that the structure (I) of the iron pigment pulcherrimin, as postulated by Kluyver *et al.*,[3] must be incorrect and further degradative evidence was therefore sought.

Pulcherriminic acid, the parent metal-free compound, has now been found to yield a dimethyl ester, $C_{14}H_{24}O_4N_2$, which appears to contain two fewer hydrogen atoms than would be demanded on the original formulation. A reconsideration of all previous analyses in this connection shows that a molecular formula $C_{12}H_{20}O_4N_2$ is at least as acceptable as $C_{12}H_{22}O_4N_2$ suggested earlier [1] (see Table).

Compound	Formula	Required (%) C	H	N	Found (%) C	H	N
Pulcherriminic acid	$C_{12}H_{22}O_4N_2$	55·8	8·5	10·8	56·1	8·3	10·8
	$C_{12}H_{20}O_4N_2$	56·2	7·8	10·9			
Morpholine salt	$C_{20}H_{40}O_6N_4$	55·6	9·3	12·9	55·6	8·9	12·7
	$C_{20}H_{38}O_6N_4$	55·8	8·8	13·1			
*cyclo*Hexylamine salt	$C_{24}H_{48}O_4N_4$	63·2	10·5	12·3	63·3	10·2	12·4
	$C_{24}H_{46}O_4N_4$	63·5	10·1	12·3			

Comparison of the infrared absorption spectra of dimethyl pulcherriminate and aspergillic acid [4] (III), in the light of what is known of their chemistry, reveals that the main features of the two spectra show a remarkable similarity. Both compounds have strong bands at 2905 cm.$^{-1}$ around 1650 cm.$^{-1}$ [1675 cm.$^{-1}$ in aspergillic acid and 1645 cm.$^{-1}$ in dimethyl pulcherriminate (carbonyl stretching frequencies)], 1600, 1480, 1400 cm.$^{-1}$ (these three bands are all common to pyrazine), 1170, 820, 780, and 740 cm.$^{-1}$. The major differences consist in strong bands at 1258 and 1020 cm.$^{-1}$ in the spectrum of dimethyl pulcherriminate which are either absent from, or much weaker in, the spectrum of aspergillic acid. These bands are typical of aromatic ethers and point to the presence of a =C–O– grouping in dimethyl pulcherriminate. It is concluded, therefore, that aspergillic acid and dimethyl pulcherriminate possess several major structural features in common and, in particular, that dimethyl pulcherriminate possesses essentially a pyrazine rather than a piperazine nucleus.

When pulcherriminic acid was reduced with red phosphorus and iodine (cf. Dutcher [5]), a new compound, dideoxypulcherriminic acid, $C_{12}H_{20}O_2N_2$, was formed. This reaction is obviously comparable with the production of deoxyaspergillic acid (IV) from the antibiotic (III) itself. The new compound must, when account is taken of previous structural

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[1] Cook and Slater, *J. Inst. Brewing*, 1954, **60**, 213.

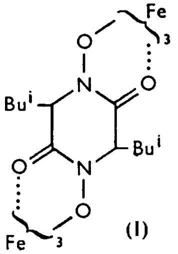
[2] Cook and Slater, preceding paper.

[3] Kluyver, van Triet, and van der Walt, *Proc. Nat. Acad. Sci. U.S.A.*, 1953, **39**, 583.

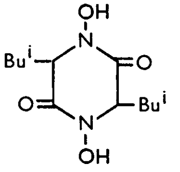
[4] Gore and Petersen, *Ann. N.Y. Acad. Sci.*, 1949, **51**, 924.

[5] Dutcher, *J. Biol. Chem.*, 1947, **171**, 321.

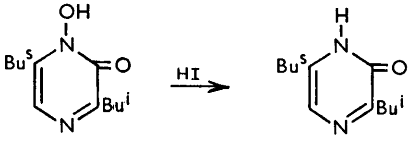
evidence regarding pulcherrimin, be formulated as (VIII), for only on further (catalytic) reduction is it converted into leucine anhydride (IX) which would almost inevitably be the



(I)

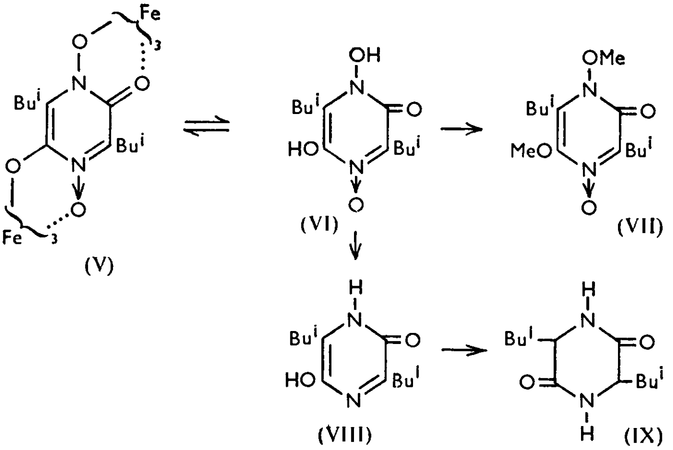


(II)



(III) (IV)

direct (phosphorus-iodine) reduction product of (II), the previous formulation for pulcherriminic acid. The infrared absorption spectrum of compound (VIII) is consistent with this structure.



All these facts are accommodated by structure (VI) for pulcherriminic acid, the corresponding ester being (VII) and pulcherrimin itself (V).

EXPERIMENTAL

Dimethyl Pulcherriminate.—Pulcherriminic acid (1 g.) was treated with a small excess of ethereal diazomethane. Evaporation of the solution followed by distillation yielded a syrup (0·7 g.), b. p. 110—120°/10 mm., which crystallised. Recrystallisation from light petroleum gave the *dimethyl ester*, m. p. 81° [Found: C, 59·0, 58·9; H, 8·6, 8·5; N, 9·7, 9·6; MeO, 21·3, 21·8%; *M* (Rast), 250. $C_{14}H_{24}O_4N_2$ requires C, 59·2; H, 8·5; N, 9·7; 2MeO, 21·8%; *M*, 284]. Light absorption: in EtOH, $\lambda_{max.}$ 233, 289, 354 mμ (ε 24,400, 6250, 7350). This compound exhibits maximal absorption at the following wavelengths in the infrared region: 3·02w, 3·44s, 4·32w, 6·08s, 6·25s, 6·58w, 6·75s, 7·14s, 7·33s, 7·51m, 7·95s, 8·37m, 8·60m, 8·74m, 9·11m, 9·20w, 9·80s, 10·21s, 10·48w, 10·82w, 10·95m, 11·10s, 12·16m, 13·29m, 13·81m, and 14·65w μ, where s = strong, m = medium, w = weak.

Dideoxypulcherriminic Acid.—A solution of pulcherriminic acid (0·5 g.) in acetic acid (20 c.c.) was added to red phosphorus (0·3 g.), iodine (0·1 g.), and acetic acid (10 c.c.) in a 50-c.c. round-bottomed flask. After being heated under reflux for 3 hr., the hot solution was filtered into cold water (80 c.c.) containing sodium metabisulphite (1 g.). Recrystallisation of the flocculent, flesh-coloured precipitate from methanol gave *dideoxypulcherriminic acid* (0·24 g., 55%), decomp. 315°, fine needles [Found: C, 64·3, 64·3; H, 8·7, 8·5; N, 12·2, 12·4%; *M* (Rast), 233. $C_{12}H_{20}O_2N_2$ requires C, 64·3; H, 8·9; N, 12·5%; *M*, 224]. Light absorption: in EtOH, $\lambda_{max.}$ 278 mμ (ε 13,250). Infrared maxima: 3·13s, 3·32s, 3·37s, 3·42s, 3·48m, 5·95s, 6·10s, 6·58w, 6·75s, 7·14s, 7·32s, 7·69m, 7·81m, 7·91w, 8·36w, 8·72m, 9·10w, 9·30w, 10·17w, 10·41w, 10·81s, 11·22w, 12·50s, and 13·31s μ. It gives no colour with ferric chloride solution, does not give a 2 : 4-dinitrophenylhydrazone, is insoluble in aqueous sodium hydrogen carbonate, and does not react with ethereal diazomethane.

Hydrogenation. A solution of dideoxypulcherriminic acid (0·025 g.) in methanol (50 c.c.) with Adams platinum catalyst (0·05 g.) was shaken with hydrogen for 3 hr. After filtration and evaporation to 5 c.c. small needles (0·015 g.) separated, having m. p. 269° alone or on admixture with authentic 2 : 5-di*iso*butyl-3 : 6-dioxopiperazine (m. p. 269°).

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