797. *Pulcherrimin: A Synthesis of* 1 : 4-*Dihydroxy*-2 : 5-*dioxopiperazines.**

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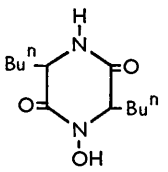
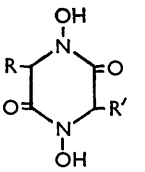
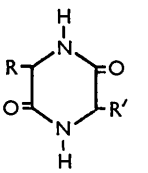
Following earlier studies,[1,2,3] representative α-hydroxyamino-acids, prepared by a new method, were converted into the corresponding substituted 1 : 4-dihydroxy-2 : 5-dioxopiperazines (II). However, 2 : 5-di*iso*butyl-1 : 4-dihydroxy-3 : 6-dioxopiperazine (II; R = R′ = Bu^i) proved, contrary to the earlier indications, to be different from the parent acid [3] of the yeast pigment pulcherrimin, the structure of which accordingly requires revision.

Van der Walt [1] and, in particular, Kluyver, van Triet, and van der Walt [2] suggested that pulcherrimin, a pigment produced by the yeast *Candida pulcherrima* (Lindner) Windisch, was an iron complex of 2 : 5-di*iso*butyl-1 : 4-dihydroxy-3 : 6-dioxopiperazine. This suggestion was supported by later more detailed work,[3] and synthesis of the pigment was therefore undertaken. As no synthetic compounds of this type had been reported, it was first desirable to devise a general synthesis of substituted 1 : 4-dihydroxy-2 : 5-dioxopiperazines. This was accomplished by a route which paralleled the synthesis of 2 : 5-dioxopiperazines from (bromoacylamino)-esters,[4] but which started from relevant α-hydroxyamino-acids instead of α-amino-acids.

Although individual α-hydroxyamino-acids have from time to time been prepared [5] the methods usually proved troublesome. In addition, little is known of the general nature of these compounds. The required intermediates were, however, in the present work obtained conveniently in about 50% yield by treating the appropriate α-bromo-acid with methanolic hydroxylamine. α-Hydroxyamino-*iso*valeric, -hexanoic, and -γ-methylvaleric acids were prepared in this way. These compounds proved to be weak acids without marked basic properties. They were strong reducing agents and on oxidation afforded


(I)


(II)


(III)

the lower homologous aldehyde. Reduction gave the corresponding amino-acid together with about 10% of the corresponding dioxopiperazine (III; R = R′ = Pr^i, Bu^n, or Bu^i). Pyrolysis yielded a mixture of products including ammonia, carbon dioxide, the corresponding amino-acid, and the lower homologous aldehyde. Attempts to prepare the *p*-bromophenacyl, *p*-nitrobenzyl, and *p*-phenylphenacyl esters by the usual techniques were unsuccessful but the methyl and the *n*-butyl esters were obtained crystalline whilst the ethyl esters were high-boiling liquids. Diazomethane reacted rapidly with α-hydroxyamino-γ-methylvaleric acid and gave by *N*-methylation as well as esterification methyl α-(*N*-hydroxy-*N*-methylamino)-γ-methylvalerate.

The methyl α-hydroxyamino-esters were more basic than the parent acids and possessed similar strong reducing properties. Methyl 2-hydroxyami ohexanoate was characterised

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[1] Van der Walt, *Proefsch. Tech. Hoges., Delft*, 1952.
[2] Kluyver, van Triet, and van der Walt, *Proc. Nat. Acad. Sci., U.S.A.*, 1953, **39**, 583.
[3] Cook and Slater, *J. Inst. Brewing*, 1954, **60**, 213.
[4] Dunn, Gallagher, Newbold, and Spring, *J.*, 1949, S130.
[5] Miller and Plöchl, *Ber.*, 1892, **25**, 2020; Traube, *ibid.*, 1895, **28**, 2300; Steiger, *J. Biol. Chem.*, 1944, **153**, 691; Snow, *J.*, 1954, 2594.

as its crystalline benzoyl derivative and as a low-melting chloroacetyl derivative. Other acyl derivatives were prepared as syrups. All these acyl compounds gave red colours with methanolic ferric chloride indicating that *N*- and not *O*-acylation had taken place.

Treatment of methyl 2-(*N*-2-bromohexanoyl-*N*-hydroxyamino)hexanoate with ammonia gave a compound, $C_{12}H_{22}O_3N_2$, which can be reasonably formulated only as (I) (cf. Dunn *et al.*[4]). Parallel reactions between hydroxylamine and methyl 2-(*N*-2-bromohexanoyl-*N*-hydroxyamino)hexanoate or methyl α-[*N*-(α-bromo-γ-methylvaleryl-*N*-hydroxyamino)]-γ-methylvalerate gave compounds which, by analogy in conjunction with the analytical evidence, are acceptably formulated as (II; R = R′ = Bu^n or Bu^i). Similarly the action of hydroxylamine on methyl 2-(*N*-chloroacetyl-*N*-hydroxyamino)-hexanoate or the γ-methylvaleryl analogue gave (II; R = H, R′ = Bu^n or Bu^i).

These representations are further supported by the fact that the compounds (II; R = R′ = Bu^n or Bu^i) give on reduction the corresponding 2 : 5-dioxopiperazines (III; R = R′ = Bu^n or Bu^i). Moreover, these compounds are weakly acidic, as would be expected, and analysis of representative methyl esters showed that each of the latter contained two methoxyl groups. In common with all hydroxamic acids, the 1 : 4-dihydroxy-2 : 5-dioxopiperazines give red colours with methanolic ferric chloride and insoluble copper complexes with methanolic cupric acetate.

2 : 5-Di*iso*butyl-1 : 4-dihydroxy-3 : 6-dioxopiperazine so synthesised differed markedly from pulcherriminic acid, the parent acid of pulcherrimin,[3] despite earlier degradative evidence. For instance, it has m. p. 253° (decomp.) whereas pulcherriminic acid has m. p. 171°. Unlike the compound from natural sources, it is not readily methylated by diazomethane, fails to give characteristic *cyclo*hexylamine or morpholine salts, and gives no precipitate but only a red colour with methanolic ferric chloride. For these reasons the structure previously given for pulcherriminic acid and for pulcherrimin [2] must be rejected.

Experimental

*α-Hydroxyamino*iso*valeric Acid.*—α-Bromo*iso*valeric acid (40 g.) was neutralised at 0° with methanolic sodium methoxide and then treated with a solution of hydroxylamine prepared as follows: Hydroxylamine hydrochloride (27·6 g.) was dissolved in warm methanol (200 c.c.) and treated with methanolic sodium methoxide from sodium (9·2 g.) and methanol (100 c.c.) with cooling; the mixture was cooled in ice and the precipitated sodium chloride removed. The mixture was heated under reflux overnight, then evaporated, and the residue treated with water. Recrystallisation of the insoluble product from water gave *α-hydroxyamino*iso*valeric acid* (2·7 g., 10%), m. p. 204° (decomp.) (Found: C, 45·4; H, 8·4; N, 10·7. $C_5H_{11}O_3N$ requires C, 45·2; H, 8·3; N, 10·5%). Similarly were obtained *2-hydroxyaminohexanoic acid* (46%), prisms, m. p. 161—162° (decomp.), from aqueous ethanol (Found: C, 49·3; H, 9·2; N, 9·4. $C_6H_{13}O_3N$ requires C, 49·0; H, 8·8; N, 9·5%), and *α-hydroxyamino-γ-methylvaleric acid* (44%) (from aqueous ethanol), plates, m. p. 155° (decomp.) (Found: C, 48·9; H, 8·8; N, 9·5%).

Reduction. Zinc dust (0·3 g.) was added during 1 hr. to a solution of α-hydroxyamino-γ-methylvaleric acid (0·5 g.) in acetic acid (10 c.c.) heated under reflux. The solution was filtered, diluted with water (10 c.c.), and adjusted to pH 6 with aqueous ammonia. Recrystallisation of the product (0·05 g.) from water gave needles, m. p. 268° alone or on admixture with 2 : 5-di*iso*butyl-3 : 6-dioxopiperazine. The residual acetic acid solution above was shown by paper chromatography to contain leucine.

Oxidation. The same hydroxyamino-acid (0·5 g.) in saturated aqueous sodium hydrogen carbonate was treated with a slight excess of aqueous potassium permanganate. The solution was distilled into aqueous 2 : 4-dinitrophenylhydrazine hydrochloride, and the dinitrophenylhydrazone was chromatographed in benzene on alumina. Recrystallisation of the product from ethanol gave *iso*valeraldehyde 2 : 4-dinitrophenylhydrazone (0·2 g.) as plates, m. p. and mixed m. p. 123°.

Reaction with diazomethane. The same hydroxyamino-acid (0·5 g.) with diazomethane (0·6 g.) in ether gave *methyl* α-(N-*hydroxy*-N-*methylamino*)-*γ-methylvalerate* (0·2 g.) as a colourless oil, b. p. 104°/6 mm. (Found: C, 55·2; H, 9·6; N, 7·6; MeO, 19·4. $C_8H_{17}O_3N$ requires C, 54·9; H, 9·7; N, 8·0; MeO, 17·7%).

Methyl 2-Hydroxyaminohexanoate.—2-Hydroxyaminohexanoic acid (10 g.) was suspended

in methanol (50 c.c.) and was treated cautiously, with stirring, with concentrated sulphuric acid (25 c.c.). The whole was set aside overnight. The solution was poured into water (200 c.c.), and excess of solid sodium carbonate added. The *methyl ester* was extracted with ether and was obtained as an almost colourless, crystalline solid (10·6 g., 96·3%). Recrystallised from light petroleum it formed needles, m. p. 55° (Found: C, 52·2; H, 9·3; N, 8·8. $C_7H_{15}O_3N$ requires C, 52·1; H, 9·3; N, 8·7). The following were prepared similarly: *Methyl* (64%), needles, m. p. 51°, from light petroleum (Found: C, 52·2; H, 9·1; N, 8·8%), and n-*butyl* α-*hydroxyamino*-γ-*methylvalerate* (from aqueous ethanol), needles, m. p. 60° (Found: C, 59·3; H, 10·5; N, 6·4. $C_{10}H_{21}O_3N$ requires C, 59·1; H, 10·4; N, 6·9%); *ethyl*, b. p. 90—95°/0·2 mm. (Found: C, 54·7; H, 9·7; N, 7·7. $C_8H_{17}O_3N$ requires C, 54·8; H, 9·7; N, 8·0%), and n-*butyl* 2-*hydroxyaminohexanoate*, b. p. 110—120°/0·1 mm. (Found: C, 59·4; H, 10·4; N, 6·6%); *ethyl* α-*hydroxyamino*-γ-*methylvalerate*, b. p. 90—95°/0·2 mm. (Found: C, 54·9; H, 9·7; N, 7·7%).

Acylation. Methyl 2-hydroxyaminohexanoate (0·5 g.) was boiled with acetic anhydride (0·5 c.c.) for 5 min. Distillation gave the *acetyl derivative* (0·2 g.), b. p. 104—104°/1·5 mm. (Found: C, 53·0; H, 8·5; N, 6·8. $C_9H_{17}O_4N$ requires C, 53·2; H, 8·4; N, 6·9%).

Methyl 2-hydroxyaminohexanoate (1 g.) in dry chloroform (10 c.c.) was treated with benzoyl chloride (0·5 c.c.) and set aside for 30 min. The solution was shaken successively with aqueous sodium hydrogen carbonate, dilute hydrochloric acid, and water until neutral. Evaporation of the chloroform gave the *benzoyl derivative* (0·4 g.) which recrystallised from light petroleum–benzene (10 : 1 v/v) in needles, m. p. 76° (Found: C, 63·2; H, 7·3; N, 5·1. $C_{14}H_{19}O_4N$ requires C, 63·4; H, 7·2; N, 5·2%). The following were prepared similarly: *Methyl* 2-(N-*chloroacetyl*-N-*hydroxyamino*)*hexanoate* which recrystallised from light petroleum in needles, m. p. 55° (Found: C, 45·6; H, 6·9; N, 5·3; Cl, 14·4. $C_9H_{16}O_4NCl$ requires C, 45·4; H, 6·7; N, 5·9; Cl, 14·9%); *methyl* α-(N-*chloroacetyl*-N-*hydroxyamino*)-γ-*methylvalerate*, m. p. 81° (Found: C, 45·2; H, 6·8; N, 5·7; Cl, 15·0%); *methyl* 2-(N-2-*bromohexanoyl*-N-*hydroxyamino*)*hexanoate*, b. p. 125—130°/0·01 mm. (Found: C, 46·3; H, 7·3; N, 4·2; Br, 24·0. $C_{13}H_{24}O_4NBr$ requires C, 46·2; H, 7·1; N, 4·1; Br, 23·7%); and *methyl* α-[N-(α-*bromo*-γ-*methylvaleryl*)-N-*hydroxyamino*]-γ-*methylvalerate*, b. p. 135—140°/10^{-3} mm. (Found: C, 46·2; H, 7·3; N, 4·0; Br, 24·6%). All these acyl derivatives gave red colours with methanolic ferric chloride.

2 : 5-*Di*-n-*butyl*-1-*hydroxy*-3 : 6-*dioxopiperazine*.—Methyl 2-(*N*-2-bromohexanoyl-*N*-hydroxyamino)hexanoate (0·4 g.) and saturated ethanolic ammonia (15 c.c.) were mixed and kept for 48 hr. Dilution with water followed by recrystallisation of the product (0·2 g.) from ethyl acetate gave the *piperazine* as plates, m. p. 187—190° (decomp.) (Found: C, 59·6; H, 9·1; N, 11·6. $C_{12}H_{22}O_3N_2$ requires C, 59·5; H, 9·1; N, 11·6%).

3-n-*Butyl*-1 : 4-*dihydroxy*-2 : 5-*dioxopiperazine*.—Methyl 2-(*N*-chloroacetyl-*N*-hydroxyamino)-hexanoate (1 g.) was kept with excess of methanolic hydroxylamine overnight; the *piperazine* (0·09 g.) separated. Evaporation of the mother-liquor gave a further crop (0·09 g.). Recrystallisation from ethyl acetate gave needles, m. p. 214° (decomp.) (Found: C, 47·6; H, 6·8; N, 13·4. $C_8H_{14}O_4N_2$ requires C, 47·6; H, 6·9; N, 13·8%). The following were prepared similarly: 3-iso*butyl*-1 : 4-*dihydroxy*-2 : 5-*dioxopiperazine* (from ethanol), needles, m. p. 235—236° (decomp.) (Found: C, 47·4; H, 6·9; N, 13·8%), and 2 : 5-*di*-n-*butyl*- (from ethyl acetate), needles, m. p. 232—233° (decomp.) (Found: C, 55·9; H, 8·3; N, 10·8. $C_{12}H_{22}O_4N_2$ requires C, 55·8; H, 8·5; N, 10·8%), and 2 : 5-*di*iso*butyl*-1 : 4-*dihydroxy*-3 : 6-*dioxopiperazine* (from ethanol), needles, m. p. 253° (decomp.) (Found: C, 55·3; H, 8·6; N, 10·9%). All these gave red colours with methanolic ferric chloride and insoluble copper complexes with methanolic cupric acetate.

Reduction. 2 : 5-Di-*n*-butyl-1 : 4-dihydroxy-3 : 6-dioxopiperazine (0·1 g.) was heated under reflux with acetic acid (10 c.c.). Zinc dust (0·15 g.) was added during 1 hr. and the solution heated for a further 2 hr. On cooling and dilution with water, 2 : 5-di-*n*-butyl-3 : 6-dioxopiperazine (0·03 g.), m. p. 269°, separated.

3-n-*Butyl*-1 : 4-*dimethoxy*-2 : 5-*dioxopiperazine*.—3-*n*-Butyl-1 : 4-dihydroxy-2 : 5-dioxopiperazine (0·1 g.) was set aside overnight with excess of ethereal diazomethane, the solid dissolving. Evaporation gave the *ester* (0·11 g.) which crystallised from *cyclo*hexane as prisms, m. p. 98° (Found: 52·4; H, 8·0; N, 12·0; MeO, 21·9. $C_{10}H_{18}O_4N_2$ requires C, 52·2; H, 7·8; N, 12·2; 2MeO, 26·9%). 3-iso*Butyl*-1 : 4-*dimethoxy*-2 : 5-*dioxopiperazine*, m. p. 115° (Found: C, 52·6; H, 7·9; N, 12·2; MeO, 22·9%), was prepared similarly.

2 : 5-*Di*iso*butyl*-1 : 4-*dimethoxy*-3 : 6-*dioxopiperazine*.—2 : 5-Di*iso*butyl-1 : 4-dihydroxy-3 : 6-dioxopiperazine (0·05 g.) in ethanol (100 c.c.) was treated with excess of ethereal diazomethane. Evaporation of the solution and recrystallisation of the product from light petroleum (b. p.

60—80°) gave the *ether* as plates (0·02 g.), m. p. 130° (Found: C, 58·7; H, 9·1; N, 9·9. $C_{14}H_{26}O_4N_2$ requires C, 58·7; H, 9·1; N, 9·8%).

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