

506. *The Structure of Pyoluteorin.*

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Spectroscopic and chemical studies lead to the structure (VI) for pyoluteorin from *Pseudomonas aeruginosa*.

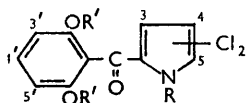
PYOLUTEORIN, the antibacterial agent produced by certain strains of the bacterium *Pseudomonas aeruginosa*,¹ was shown by Takeda^{2,3} to have the partial structure (I), in which the chlorine substitution pattern in the pyrrole ring was not established. Since pyoluteorin itself could be *N*-methylated with diazomethane to yield the *OON*-trimethyl derivative (II), whereas didechloropyoluteorin² (III) afforded only an *OO*-dimethyl derivative (IV) with this reagent, one of the chlorine functions was tentatively placed³ in the 5-position. In agreement with this difference in NH reactivity, we find that, in the presence of alkali, the ultraviolet spectrum of *OO*-diacetylpyoluteorin shows a strong bathochromic shift, in contrast to the unchanged spectrum of didechloro-*OO*-dimethylpyoluteorin (IV).

¹ Takeda, *J. Ferment. Technol., Osaka*, 1958, **36**, 281.

² Takeda, *Bull. Agric. Chem. Soc. Japan*, 1959, **23**, 126.

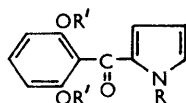
³ Takeda, *J. Amer. Chem. Soc.*, 1958, **80**, 4749; *Bull. Agric. Chem. Soc. Japan*, 1959, **23**, 165.

The proton magnetic resonance (p.m.r.) spectrum (in deuteriochloroform) of trimethylpyoluteorin (II) shows the pyrrole hydrogen as a singlet at 3.57 τ , together with resonances due to *O*-methyl (6.26 τ), *N*-methyl (5.91 τ), and the benzenoid hydrogens H-3', H-4', and H-5', which will be mentioned later. Catalytic hydrogenolysis² of the chlorine atoms yields didechlorotrimethylpyoluteorin (V), in which the methyl and benzenoid hydrogen



(I : R = R' = H)

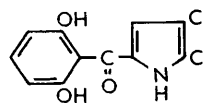
(II : R = R' = Me)



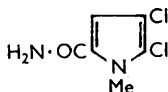
(III : R = R' = H)

(IV : R = H, R' = Me)

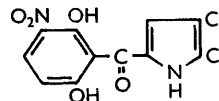
(V : R = R' = Me)



(VI)



(VII)



(VIII)

resonances are unchanged, whilst the three pyrrole hydrogens, mutually spin-coupled, appear as multiplets centred at 3.93, 3.51, and 3.14 τ . In *N*-methylpyrrole itself (in the same solvent), H-2 and H-5 resonate at 3.44 τ , and H-3 and H-4 at 3.89 τ , and the presence of an electron-withdrawing dimethoxybenzoyl substituent in the 2-position, while scarcely affecting H-4, would strongly deshield H-3 and, to a somewhat lesser extent, H-5.⁴ Consequently, the resonances at 3.93, 3.51, and 3.14 τ in the didechloro-compound (V) can only be due to H-4, H-3, and H-5, respectively. Since the introduction of halogen substituents into pyrrole rings,⁴ as in other systems,⁵ causes only minor shifts in the resonance frequencies of the remaining hydrogens, the H-3 quartet at 3.51 τ in (V) corresponds to the original pyrrole hydrogen singlet at 3.57 τ in the chloro-compound (II), leading to structure (VI) for pyoluteorin. Coupling constants ($J_{3,4} = 4.1$, $J_{3,5} = 1.7$, $J_{4,5} = 2.5$ c./sec.) deduced from the quartets due to H-3 and H-4, and the pattern due to H-5 in the spectrum of the didechloro-compound (V), are in agreement with those found⁴ in other pyrrole systems, and confirm the proton assignments.

An interesting consequence of the planarity or near-planarity of the conjugated chromophore in compounds (II) and (V) is the non-identity in the p.m.r. spectra of the benzenoid protons H-3' and H-5'. Although these protons do not couple appreciably with each other, they resonate at slightly different frequencies (as doublets centred at 3.41 and 3.42 τ), and have different coupling constants (7.5, 9.1 c./sec.) with H-4' itself a quartet (centred at 2.68 τ , $J = 7.5, 9.1$ c./sec.).

Chemical confirmation of structure (VI) for pyoluteorin followed from cleavage of its *OO**N*-trimethyl-derivative (II), under modified Haller-Bauer conditions⁶ using sodamide in liquid ammonia, to yield 1,3-dimethoxybenzene and a dichloro-1-methylpyrrole-2-carboxamide. The ring hydrogen singlet at 3.06 τ in the p.m.r. spectrum (in acetone) of this amide does not unequivocally distinguish between the 4,5- and 3,4-dichloro-structures. However, the amide was identical with authentic 4,5-dichloro-1-methylpyrrole-2-carboxamide (VII) prepared from methyl 4,5-dichloro-1-methylpyrrole-2-carboxylate⁴ by way of the acid and acid chloride, and comparison of the corresponding acids confirmed this identity.

The reaction of pyoluteorin with nitric acid in acetic acid gave a yellow mononitration product. In view of its solubility in sodium hydrogen carbonate, this is probably the 3'-nitro-derivative (VIII) rather than the alternative 3-nitro-compound which would not

⁴ Hodge and Rickards, unpublished work.

⁵ Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 63; White, in "Physical Methods in Heterocyclic Chemistry," Academic Press, London, 1963, Vol. II, p. 103.

⁶ Hamlin and Weston, *Org. Reactions*, 1957, 9, 1.

be expected⁷ to show such increased acidity relative to pyoluteorin itself. Treatment of either pyoluteorin or the amide (VII) with fuming nitric acid or chromic acid gave no trace of the chloromaleimide desired for structural investigations, and since halogenomaleimides formed by oxidation of halogenopyrroles are of doubtful structural significance,⁸ this oxidative degradation was not pursued.

The benzylation of the 2-lithio-derivative of 1,3-dimethoxybenzene and of the potassium salt of 2,5-dihydro-1,3-dimethoxybenzene were studied as possible models for the synthesis of the pyoluteorin system (VI), but were unsuccessful. However, reaction of pyrrolylmagnesium bromide with 2,6-dimethoxybenzoyl chloride gave, in good yield, didechloro-*OO*-dimethylpyoluteorin (IV), which on methylation with methyl iodide in the presence of potassium carbonate afforded didechloro-*OON*-trimethylpyoluteorin (V). Treatment of the *OO*-dimethyl compound (IV) with demethylating agents under various conditions resulted either in recovery of starting material or fragmentation of the molecule by a reverse Friedel-Crafts mechanism. Attempted 4,5-dichlorination of the heterocyclic ring in the ketones (IV) and (V), using chlorine in acetic acid (cf. ref. 4), gave mixtures as a result of the comparable reactivities of the two aromatic rings present.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. Infrared (i.r.) spectra were measured on a Unicam S.P. 200 spectrophotometer, and ultraviolet (u.v.) spectra on a Unicam S.P. 500 spectrophotometer. P.m.r. spectra were recorded for ca. 11% solutions, containing tetramethylsilane as internal reference, on a Varian Associates A60 machine.

OO-*Diacetylpyoluteorin*.—Prepared according to Takeda,² this had m. p. 204–206°, λ_{infl} 248 m μ , λ_{max} 311 m μ (log ϵ 3.71, 4.17) in neutral ethanol, λ_{max} 335 m μ (log ϵ 4.30) upon addition of sodium hydroxide.

Cleavage of OON-Trimethylpyoluteorin with Sodamide.—To a stirred suspension of sodamide (from sodium, 0.5 g.) in liquid ammonia (150 ml.) was added trimethylpyoluteorin² (1.0 g.) in ethylene glycol dimethyl ether (2 ml.). After 1 hr., water (150 ml.) was added and the ammonia evaporated. The crystalline precipitate (428 mg.) was filtered off and the filtrate extracted with ether. The dried extract was evaporated to a residue which, after washing with light petroleum (b. p. 40–60°), was combined with the earlier precipitate, to give the dichloro-1-methylpyrrole-2-carboxamide (590 mg., 96%), m. p. 160.5–163° (from ether-hexane), λ_{max} 274 m μ (log ϵ 3.83) [Found: C, 37.4, 37.7; H, 3.2, 3.3; Cl, 36.1; N, 13.5%; *M* (mass spectroscopy), 193. Calc. for C₆H₆Cl₂N₂O: C, 37.3; H, 3.1; Cl, 36.8; N, 14.5%; *M*, 193], identical (mixed m. p. and i.r. spectrum) with 4,5-dichloro-1-methylpyrrole-2-carboxamide.

The light-petroleum washings, on evaporation, afforded 1,3-dimethoxybenzene (320 mg., 73%) as an oil, purified by distillation and identified by i.r. and u.v. spectroscopy.

Hydrolysis of the Dichloro-1-methylpyrrole-2-carboxamide.—The amide (113 mg.) in ethanolic potassium hydroxide (5 ml., 10%) was refluxed for 3 hr. The solution was diluted with sodium chloride solution, washed with ether, and acidified. Ether extraction and recovery afforded the dichloro-1-methylpyrrole-2-carboxylic acid (71 mg., 63%), needles, m. p. 170–185° (decomp.) (from ether-hexane) (Found: C, 36.9; H, 2.4; N, 7.1. Calc. for C₆H₅Cl₂NO₂: C, 37.1; H, 2.6; N, 7.2%), identical (mixed m. p. and i.r. spectrum) with 4,5-dichloro-1-methylpyrrole-2-carboxylic acid.

3'-Nitropyloluteorin.—Concentrated nitric acid (0.3 ml.) was added to pyoluteorin (63 mg.) and urea (5 mg.) in acetic acid (1 ml.) at 0°. After 30 min., the mixture was poured on to ice and extracted with ethyl acetate. The extract was dried and evaporated, and the ether-soluble fraction of the residue was chromatographed on anhydrous magnesium sulphate. The eluate was washed with aqueous potassium hydrogen carbonate, and the washings acidified and re-extracted with ether. Recovery gave 3'-nitropyloluteorin (VIII) (40 mg.), yellow crystals, m. p. 226–228° (from benzene), λ_{infl} 245 m μ , λ_{max} 310 m μ (log ϵ 2.31, 4.36) in neutral ethanol, changing upon addition of sodium hydroxide to λ_{max} 325, 400 m μ (log ϵ 4.33, 4.36), ν_{max} (in Nujol) 3290, 3170 (OH), 1620 (H-bonded carbonyl), and 1560 cm.⁻¹ (NO₂) (Found: C, 42.4; H, 2.2; N, 8.7. C₁₁H₆Cl₂N₂O₅ requires C, 41.6; H, 1.9; N, 8.8%).

⁷ Cf. Rinkes, *Rec. Trav. chim.*, 1934, **53**, 1167.

⁸ Rinkes, *Rec. Trav. chim.*, 1941, **60**, 303, and references therein.

4,5-Dichloro-1-methylpyrrole-2-carboxylic Acid.—Methyl 4,5-dichloro-1-methylpyrrole-2-carboxylate⁴ (82 mg.) was refluxed in methanolic potassium hydroxide (10 ml.; 10%) for 4 hr. Acidification and ether-extraction afforded the *acid* (74 mg., 98%), needles, m. p. 174—186° (decomp.) (from hexane-ether), ν_{\max} . (in Nujol) 3300—2400 and 1675 cm^{-1} (Co_2H) (Found: C, 37.2; H, 2.4; N, 7.2. $\text{C}_8\text{H}_5\text{Cl}_2\text{NO}_2$ requires C, 37.2; H, 2.6; N, 7.2%).

4,5-Dichloro-1-methylpyrrole-2-carboxamide.—The above acid (68 mg.) was refluxed with thionyl chloride (5 ml.) for 1 hr. Evaporation of the excess of reagent left the crystalline acid chloride, which was dissolved in ether (5 ml.) and added carefully to liquid ammonia (30 ml.). After evaporation of the ammonia, water was added and the mixture extracted with ether. The recovered solid was purified by sublimation at 100°/0.1 mm., to yield long needles of the *amide* (VII) (50 mg., 74%), m. p. 160.5—162.5° (from hexane-ether), ν_{\max} . (in Nujol) 3430, 3220 (NH), 1655 (amide CO), and 1615 cm^{-1} (pyrrole) (Found: C, 37.1; H, 3.1; N, 14.2. $\text{C}_8\text{H}_6\text{Cl}_2\text{N}_2\text{O}$ requires C, 37.3; H, 3.1; N, 14.5%).

Didechloro-OO-dimethylpyoluteorin (IV).—2,6-Dimethoxybenzoyl chloride⁹ (4.3 g.) in ether (20 ml.) was added dropwise to a stirred solution of pyrrolmagnesium bromide, prepared by adding pyrrole (1.4 g.) in ether (5 ml.) to ethylmagnesium bromide (from ethyl bromide, 2.2 g.) in ether (100 ml.). After refluxing for 4 hr., the cooled mixture was added to water, the ethereal layer separated, and the aqueous layer extracted with ethyl acetate. The combined organic extracts were washed with aqueous sodium hydrogen carbonate and dried. Recovery afforded didechloro-OO-dimethylpyoluteorin (IV) (3.1 g., 55%), m. p. 193—196° (from aqueous methanol), ν_{\max} . (in Nujol) 3220 (NH) and 1612 cm^{-1} (C=O), λ_{infr} (in ethanol) 255, λ_{\max} . 297 $\text{m}\mu$ ($\log \epsilon$ 3.70, 4.19) (Found: C, 67.2; H, 5.5; N, 6.0. Calc. for $\text{C}_{13}\text{H}_{13}\text{NO}_3$: C, 67.5; H, 5.7; N, 6.1%) [lit.,² m. p. 187—188°, ν_{\max} . (in Nujol) 3230 and 1615 cm^{-1}].

Didechloro-OON-trimethylpyoluteorin (V).—Methylation of didechloro-OO-dimethylpyoluteorin (IV) (340 mg.) by refluxing with methyl iodide (10 ml.) and potassium carbonate (3 g.) for 60 hr. gave the *trimethyl compound* (V) (300 mg., 83%), prisms, m. p. 137—140° (from methanol), ν_{\max} . (in CS_2) 1639 cm^{-1} (C=O), λ_{\max} . (in ethanol) 296 $\text{m}\mu$ ($\log \epsilon$ 4.26) [lit.,² m. p. 137°, ν_{\max} . (in Nujol) 1630 cm^{-1} , λ_{\max} . (in ethanol) 295 $\text{m}\mu$ ($\log \epsilon$ 4.26)].

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⁹ Stork and Clarke, *J. Amer. Chem. Soc.*, 1961, **83**, 3114.