Pyoluteorin, a synthesis

K. BAILEY[1] AND A. H. REES[2]
Department of Chemistry, Trent University, Peterborough, Ontario

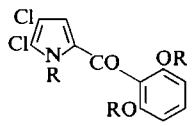
Received March 5, 1970

The synthesis of *Pyoluteorin*, an antibiotic from *Pseudomonas aeruginosa* is described. The method used produced in addition, an isomer 3,5-dichloro-2-(2′,6′-dihydroxybenzoyl)pyrrole, for which the name *isopyoluteorin* is proposed. The bromine analogue of pyoluteorin is also reported.

Canadian Journal of Chemistry, 48, 2257 (1970)

Pyoluteorin (**1**) (1, 2) is a powerful bactericide produced by certain strains of *Pseudomonas aeruginosa* (3). It is also a potent fungistat (4).

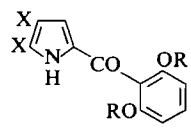
Attempts at its synthesis have devolved upon the benzoylation of a pyrrole or the pyrroylation of a benzenoid moiety.



1 R = R′ = H
2 R = Me; R′ = H
3 R = R′ = Me
4 R = H; R′ = Me
5 R = H; R′ = $PhCH_2$

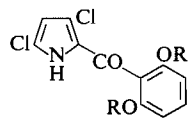
The former method (2) gave didechloro-*O*,*O*′-dimethylpyoluteorin (**6**) but not pyoluteorin. The latter (5) gave *N*,*O*,*O*′-trimethylpyoluteorin (**3**), *N*-methylpyoluteorin (**4**), and *N*-benzylpyoluteorin (**5**) but not pyoluteorin.

We reported briefly (6) that dimethylpyoluteorin (**2**) was obtained by heating 2,6-dimethoxybenzoyl chloride with sodium 4,5-dichloropyrrole-2-carboxylate and that the product could be demethylated to yield the synthetic antibiotic, 4,5-dichloro-2-(2′,6′-dihydroxybenzoyl)pyrrole, identical with the natural pyoluteorin originally reported by Takeda (1).



6 R = Me; X = H
7 R = Me; X = Br
8 R = H; X = Br

We now give full details of our synthesis. We chlorinated 2-carbomethoxypyrrole using chlorine in acetic acid or *t*-butyl hypochlorite in carbon tetrachloride, but were unable to reproduce the published results (7); in both cases a mixture of 4,5- and 3,5-dichloro derivatives was obtained which could not be completely resolved by column chromatography or fractional recrystallization. Rather than effect a tedious separation, we hydrolyzed the mixture to the corresponding acids and proceeded. By the method indicated above, we obtained a mixture of *O*,*O*′-dimethylpyoluteorin (**2**) and 3,5-dichloro-2-(2′,6′-dimethoxybenzoyl)pyrrole (*O*,*O*′-dimethylisopyoluteorin) (**9**).



9 R = Me
10 R = H

Column and thin-layer chromatography (t.l.c.) were again not efficient methods of separation but fractional crystallization yielded pure isomer **9**. Demethylation of the mixture of ethers by boron trichloride followed by t.l.c. resulted in separation of **1** from isopyoluteorin (**10**). The nuclear magnetic resonance (n.m.r.) spectra of these compounds were in agreement with their structures and followed from those of the starting dichloropyrrole esters (7). Although a pure sample of **2** was not obtained here, its spectrum was easily determined from that of its mixture with the isomeric ether **9** and that of pure **9**. Subsequent confirmation has come from the spectrum of pure **2** synthesized by another route (8).

As noted by Birch *et al.* (2), the 3′- and 5′-protons of pyoluteorin are not equivalent in the n.m.r. spectrum for although they have almost

[1]Present address: Food and Drug Directorate, Tunney's Pasture, Ottawa 3, Canada.
[2]To whom enquiries should be directed.

TABLE 1*

The n.m.r. spectra of pyoluteorin analogues and derivatives

Compound solvent	CH_3 3′,5′	4′	3	4	OH	NH
1 DMSO-d_6	3.62 d	2.96 q [7.5,8.5]	3.57†		0.50	−3.08
2 $CDCl_3$	6.25 3.43 d	2.68 q [7.5,8.5]	3.54 d [3.0]			0.0
7 $CDCl_3$	6.24 3.42 d	2.66 q [7.5,9.0]	3.52 d [2.5]			−0.15
9 $CDCl_3$	6.25 3.42 d	2.66 q [7.5,8.5]		3.93 d [3.0]†		0.3
10 DMSO-d_6	3.66 d	2.99 q [7.2,8.5]		3.72†	0.60	−2.8

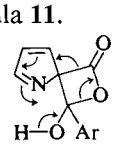
*T.M.S. internal standard; d = doublet, q = quartet; figures in brackets indicate splitting [Hz] observed: signals assigned to OH integrated for two protons and to NH (broad) for 1 proton, and disappeared on addition of D_2O.
†Sharpened or collapsed to singlet on adding D_2O.

indistinguishable chemical shifts, their coupling constants with the 4′-proton differ, J = 8.5 and 7.5 Hz. In compounds **1**, **2**, **6**, **9**, and **10** these protons give rise to more or less superimposed broad-branched doublets (Table 1). However, in certain related compounds (8) these protons have quite different chemical shifts. Although in **1** and isomer **10**, the pyrrole protons overlap with signals from the 3′- and 5′- benzenoid protons, on deuterium exchange the pyrrole proton fine doublets collapse to singlets and the signals show an obvious increase in height.

The infrared (i.r.) spectra (Nujol) of pyoluteorin and its dimethyl ether have carbonyl bands at 1633 cm^{-1} with a further characteristic band at 890 cm^{-1}. In the *iso*-series these appear at 1620 and about 915 cm^{-1} respectively while the bromine analogue **8** of pyoluteorin and its ether **7** have bands at 1635 and 895 cm^{-1}. The pyoluteorins and their ethers also show a band at about 1595 cm^{-1} but a further band at 1565 cm^{-1} which we assign to hydrogen-bonded carbonyl is only found in the free phenols.

The ultraviolet (u.v.) spectra of the pyoluteorins show a bathochromic shift from about 305 to 330 mμ on addition of alkali. However, if the analogues **1** or **10** in neutral ethanol ($\lambda_{max} \sim 305$ mμ) are made only faintly alkaline, the maximum shifts to about 365 mμ and more base must be added to bring this peak back to 330 mμ. Since the pyrrole NH is quite acidic, mesomeric mono-, di-, and trivalent anions are obviously involved.

A possible mechanism for the key reaction is indicated by formula **11**.



11

Experimental

Pyoluteorin and Isopyoluteorin Dimethyl Ethers

A mixture of dry sodium 3,5- and 4,5-dichloropyrrole-2-carboxylates (from 1.8 g acid mixture) was refluxed 75 min in 5 ml dry benzene with 2,6-dimethoxybenzoyl chloride (from 2 g acid). The solvent was then distilled off and the residue heated at 150° for 45 min and cooled. The products were exhaustively extracted with benzene and the concentrated extract adsorbed onto silica gel (120 g, containing 5% w/w water). Elution with light petroleum containing increasing amounts of ether gave the following fractions: (*i*) 410 mg oil using 15 × 100 ml eluate, 0–40% ether; (*ii*) 1.16 g mixture using 7 × 100 ml eluate, 40% ether. The third of fractions (*ii*) was evaporated giving 360 mg material. Crystallization from benzene/petrol gave *O*,*O*′-dimethylisopyoluteorin (90 mg) m.p. 204–205°, ν_{max} 3210, 1618, 1595, 1108, and 910 cm^{-1}.

Anal. Calcd. for $C_{13}H_{11}Cl_2NO_3$: C, 52.0; H, 3.7; Cl, 23.6; N, 4.7. Found: C, 52.1; H, 3.36; Cl, 23.5; N, 4.8.

The remainder of fraction (*ii*) (1.07 g) was purified by t.l.c. giving 200 mg material with λ_{max} 300 mμ, m.p. 155–185°, shown by n.m.r. spectroscopy to consist of comparable amounts of pyoluteorin and isopyoluteorin dimethyl ethers.

Fraction (*iii*) 825 mg using 50–100% ether. This was mainly 2,6-dimethoxybenzoic acid, also present as an impurity in (*ii*).

Isopyoluteorin

A solution of dimethylisopyoluteorin (40 mg) in methylene chloride (15 ml) at −70° was treated with boron trichloride (3 ml 40% w/w in methylene chloride). After 2 h the solution was allowed to warm up slowly, 20 h, when it became deep red. Excess reagent was destroyed by cautious addition of water and more solvent. The organic layer was washed with water, then with brine, dried ($MgSO_4$), and evaporated leaving isopyoluteorin (25 mg) m.p. 198–203° from benzene, ν_{max} 3350, 3240, 3130, 1620, 1565, 1017, 920 cm^{-1}.

Anal. Calcd. for $C_{11}H_{11}Cl_2NO_3$: C, 48.6; H, 2.6; Cl, 26.1; N, 5.15. Found: C, 48.6; H, 2.5; Cl, 26.2; N, 5.1.

Pyoluteorin

The mixture of the dimethyl ethers of pyoluteorin and isopyoluteorin (75 mg) was demethylated as above and the products separated by t.l.c. on silica plates using acetone/petrol (3:7). The lower band (23 mg) was the iso-compound **10** and the upper band was pyoluteorin

1 which crystallized as sulfur yellow needles (35 mg) from benzene/petrol with m.p. 177–186°, varying slightly with the rate of heating, undepressed in mixed melt with an authentic sample of m.p. 175–182°, ν_{max} 3470, 3370, 3160, 1633, 1599, 1023, 890 etc. cm^{-1}.

Anal. Calcd. for $C_{11}H_7Cl_2NO_3$: C, 48.6; H, 2.6; Cl, 26.0; N, 5.15. Found: C, 49.0; H, 2.5; Cl, 25.4; N, 5.15.

Pyoluteorin Bromine Analogue

4,5-Dibromopyrrole-2-carboxylic acid (4 g as the sodium salt) was refluxed in dry benzene (50 ml) with 2,6-dimethoxybenzoyl chloride (from 3 g acid) for 90 min. The solvent was then distilled off and the residue heated to 150° for 1 h, then extracted with three 150 ml portions of 1:1 benzene/ethyl acetate. The total extract was washed with sodium carbonate solution, then water, dried, and evaporated leaving 1 g residue. Preparative t.l.c. in 1:1 ether/petrol, three passes, gave a band at R_f 0.6 which was extracted to yield 300 mg crystalline product m.p. 200–205° from benzene/petrol.

Anal. Calcd. for $C_{13}H_{11}Br_2NO_3$: C, 40.1; H, 2.85; Br, 41.1; N, 3.6. Found: C, 40.3; H, 2.8; Br, 42.6; N, 3.9.

The n.m.r. spectrum (Table 1) confirmed the structure **7**. The mass spectrum revealed the presence of some 2-(2′,6′-dimethoxybenzoyl)-3,4,5-tribromopyrrole.

The above dimethyl ether (125 mg) in methylene chloride (10 ml), at −70° was treated with the methylene chloride solution of boron trichloride (6 ml) when a purple complex formed which rapidly turned deep red. The solution was allowed to warm up to room temperature (1 h) and left overnight then worked up as described to give 90 mg crystals which were further purified by t.l.c. in acetone/petrol 1:5. The major product, R_f 0.5, was crystallized from benzene/petrol to give yellow prisms m.p. 163–164°.

Anal. Calcd. for $C_{11}H_7Br_2NO_3$: C, 36.6; H, 1.95; Br, 44.3; N, 3.9. Found: C, 36.6; H, 2.25; Br, 44.2; N, 4.0.

The mass spectrum did not reveal any tribromoanalogue. Its cracking pattern resembled that of pyoluteorin.

We thank the National Research Council of Canada for financial support.

1. R. TAKEDA. J. Amer. Chem. Soc. **80**, 4749 (1958), Bull. Agr. Chem. Soc. Japan, **23**, 126, 165 (1959).
2. A. J. BIRCH, P. HODGE, R. W. RICKARDS, R. TAKEDA, and T. R. WATSON. J. Chem. Soc. 2641 (1964).
3. R. TAKEDA. J. Ferment. Technol. Osaka, **36**, 281 (1958).
4. (*a*) P. HODGE. Personal communication 1964. (*b*) R. J. O'CONNER, J. M. VAN DEREN, and W. A. DARLINGTON. U.S. Pat. 3 298 910 (1967).
5. J. A. ELIX and M. V. SARGENT. J. Chem. Soc. (C) 1718 (1967).
6. K. BAILEY and A. H. REES. J. Chem. Soc. (D) 1284 (1969).
7. P. HODGE and R. W. RICKARDS. J. Chem. Soc. 459 (1965).
8. G. R. BIRCHALL and A. H. REES. Unpublished observations.