THE STRUCTURE OF RP 18,631

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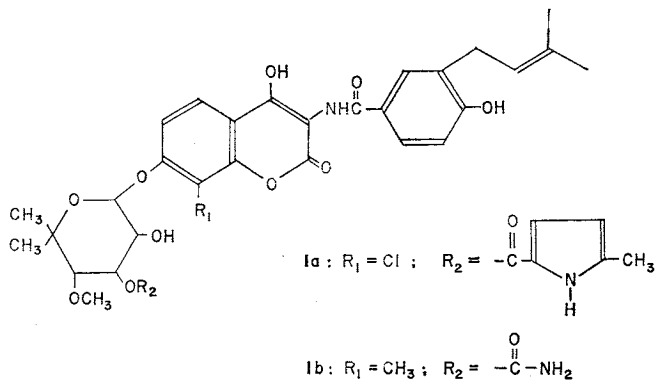
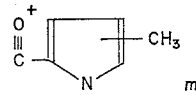
The complete structure of RP 18,631, a new chlorine-containing antibiotic related to novobiocin, has been determined using a combination of degrative and nuclear magnetic resonance techniques. Of particular interest is the long-range couplings observed in the pyrrole ring present in the molecule.

We wish to report the complete structure of RP 18,631, a new member of the novobiocin family of antibiotics. The partial structure disclosed in a British patent[1] issued to Rhône-Poulenc left the position of the chlorine atom on the coumarin ring and that of the methyl group on the pyrrole ring unspecified. This note reports our work indicating that chlorobiocin has structure **Ia**. The structure of novobiocin (**Ib**) is given for comparison purposes.



Employing a scheme similar to that used to determine the structure of novobiocin[2] (Scheme 1), we refluxed RP 18,631 in ethanol containing a trace of acetyl chloride to give sugar **II** and compound **III**.

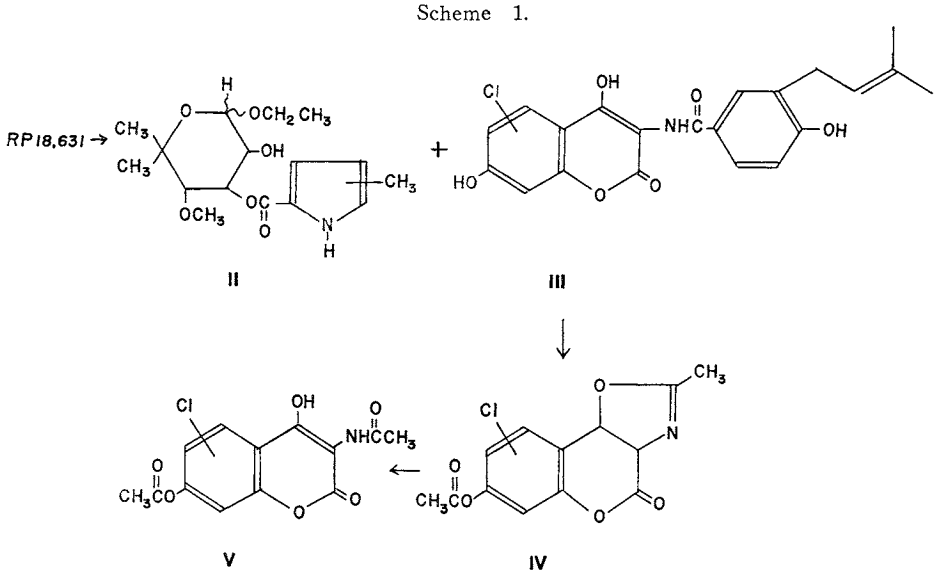
The structure of sugar **II** was determined from spectral data. High resolution mass spectroscopy indicated a molecular ion at *m/e* 327.1697, corresponding to $C_{16}H_{25}NO_6$ (327.1682). The most intense peak appeared at *m/e* 108 corresponding to the expected ion **VI**.



VI
m/e 108

Other peaks appeared at *m/e* 312 (M^+–CH_3), 282 (M^+–OEt), 269 (M^+–acetone), 182 (**VII**) and 167 (**VIII**).

Scheme 1.



The ultraviolet spectrum of II showed a maximum at 278 nm (ε=20,300). This indicates that the carbonyl group on the pyrrole ring is at the 2-position since 3-carboxylpyrroles absorb at a shorter wavelength[3].

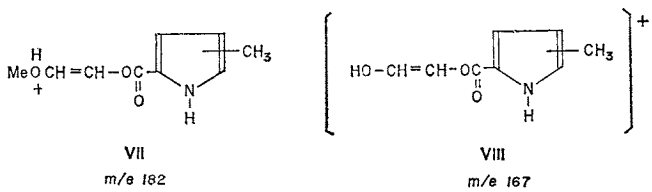
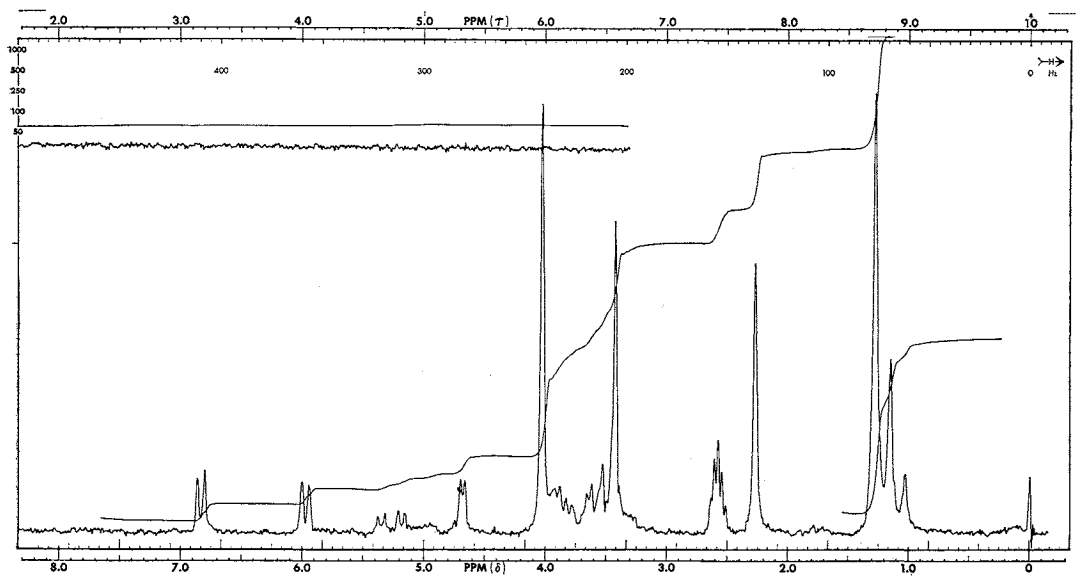


Fig. 1. NMR spectrum of II in d_6-DMSO/D_2O with TMS standard.
Upper trace is at offset of 300 cps.



The NMR spectrum of II in d_6-dimethylsulfoxide after complete exchange of the hydroxyl and the labile pyrrole protons with D_2O (steam bath, 15 minutes) is shown in Fig. 1. The chemical shifts are such that all the protons can be assigned (Table 1). Two pyrrole ring protons appear as doublets (J=3.5 cps) at 5.9 ppm and 6.7 ppm. Examination of pyrrole coupling constants given in the literature[4,5] revealed that only coupling by adjacent protons in the 3- and 4-ring positions could be larger than 3.1 cps. This requires that the methyl group be at the 5-position.

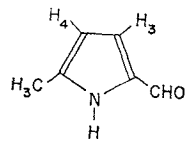
Table 1. Peak assignments for Fig. 1.

Shift	Multiplicity	Coupling constant	Integral	Assignment
1.13 ppm	triplet	7.0 cps	3 protons	ethoxyl methyl
1.22 ppm	singlet	—	6 protons	C-methyls
2.25 ppm	singlet	—	3 protons	pyrrole methyl
3.3 ppm	multiplet	—	1 proton	alpha to ether
3.40 ppm	singlet	—	3 protons	O-methyl
3.46 ppm	quartet	7.0 cps	2 protons	ethoxyl methylene
3.87 ppm	multiplet	—	1 proton	alpha to hydroxyl
4.05 ppm	—	—	—	water
4.64 ppm	doublet	2.0 cps	1 proton	alpha to ester
5.11 to 5.29	doublets	3.0 cps	1 proton	anomeric (α and β)
5.9 ppm	doublet	3.5 cps	1 proton	pyrrole ring
6.72 ppm	doublet	3.5 cps	1 proton	pyrrole ring

Note: The spectrum was run on an A 60 spectrometer at ambient temperature using deuterated dimethylsulfoxide as the solvent with tetramethylsilane as the internal reference after heating with an excess of D_2O for 15 minutes on a steam bath.

Examination of the 60-MHz spectrum of II revealed that the singlet at 2.25 ppm assigned to the pyrrole methyl is noticeably broader at half-height than is the singlet at 3.40 ppm assigned to the O-methyl group. We inferred that long-range coupling with one or more of the ring protons accounted for this.

Spin decoupling experiments at 100 MHz indicated that both pyrrole ring protons were coupled to the methyl protons with a coupling constant of 0.5±0.2 cps. Coupling *via* five chemical bonds is a known phenomenon and several examples of it are given in a review by STERNHELL[6]. Such "homoallylic" coupling constants can be as great as 2.0 cps. As an example the methyl protons of 5-methylpyrrole-2-carboxaldehyde (**IX**) are coupled to the protons at the 3- and 4-ring positions with J values of 0.67 and 0.45 cps respectively[4]. Therefore, the ester on the sugar of chlorobiocin is a 5-methylpyrrole-2-carboxylate as it is in coumermycin A_1[3].



IX

The NMR spectrum of compound III in d_6-DMSO gave a complex aromatic region. Therefore, III was converted to oxazole IV and to amide V using pyridine/acetic anhydride followed by acid hydrolysis. The NMR spectra of IV and V both showed a pair of doublets (J=8.5 cps) indicating that the ring protons must be adjacent to one another and that the chlorine atom must be at the 8-position of the coumarin ring. Spectral and analytical data for III, IV and V are consistent with the structures as written and are in agreement with the structure the French workers indicated.

After this work was completed Professor K. L. RINEHART of the Uiversity of Illinois (personal communication) was able to resolve the coumarin doublets of compound III using pyridine as solvent at 60 MHz and at 220 MHz.

Experimental

The Formation of II and III

RP 18,631* (500 mg, 0.72 mmole) was refluxed in 50 ml of methanol with 0.2 ml of acetyl chloride under nitrogen for 2 hours[2]. The solution was cooled to 20°C and poured into 125 ml of water. The resulting precipitate was taken up in hot acetone and the solution was treated with water until cloudy. The solution deposited 200 mg (0.48 mmole) or 67.7 % of a white solid, III, m.p. 217~221°C (dec).

Analysis Calcd. for $C_{21}H_{18}ClNO_6 \cdot {}^1/_2H_2O$: C 59.37, H 4.51, Cl 8.35, N 3.30
Found: C 59.32, H 4.55, Cl 8.33, N 2.99.

The high resolution mass spectrum showed a molecular ion at 415.0821 (Calculated molecular weight for $C_{21}H_{18}ClNO_6$=415.0822).

Compound II was obtained by lyophilizing the filtrate obtained from the first precipitate. The resulting white solid showed one spot by tlc and gave a mass spectral molecular ion at *m/e* 327.1697. (Calculated molecular weight=327.1682). The NMR spectrum is discussed in the text.

Oxazole (IV)

Compound III (900 mg, 2.13 mmoles) containing water of solvation was refluxed in 9 ml of pyridine with 1.8 g of acetic anhydride under nitrogen for 4 hours[2]. The mixture was cooled to 10°C and diluted to 35 ml with ice water. The pH was adjusted to 2 with 3 N HCl and the resulting precipitate was collected and washed with water. The filter cake was slurried with ether three times and the insoluble gray solid was dissolved in 75 ml of hot ethanol. Water was added to cloudiness. Gray solid precipitated on cooling, m.p. 230~232°C. The UV spectrum in ethanol had maxima at 355 nm (ε=674), 322 (9,650), 314 (10,300), 308 (10,900), 287 (11,950), 277 (9,800), 242 (10,150) and 214 (8,950) in good agreement with the oxazole obtained from novobiocin.

Analysis Calcd. for $C_{13}H_8ClNO_5$: C 53.17, H 2.74, Cl 12.08, N 4.77
Found: C 53.50, H 2.79, Cl 12.03, N 4.99.

Amide (V)

A sample of compound IV was allowed to stand at 25°C for 16 hours at pH 2 in aqueous ethanol. The white solid which precipitated melted at 256~257°C (dec). The NMR spectrum was very similar to that of the oxazole but the IR spectrum showed hydroxyl, N-H and amide carbonyl bands. The UV (EtOH) showed maxima at 313 nm (ε=24,600), 278 (21,400) and 287 (18,700).

Analysis Calcd. for $C_{13}H_{10}ClNO_6$: C 50.09, H 3.23, Cl 11.38, N 4.49
Found: C 49.90, H 3.27, Cl 10.86, N 4.33.

Note: After the work was completed we received a pre-publication copy of a manuscript by L. NINET[7] *et al.*, which reports the complete structure of chlorobiocin. Their structure is in accord with our Ia but they give no experimental evidence in that paper.

Acknowledgement

The author thanks S. MISZAK for the decoupling experiments and R. WNUK for the high resolution mass spectra.

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