260. A Structural Investigation of the Antibiotic Rubiflavin[1])

Preliminary communication[2])

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(29.X.80)

Summary

Rubiflavin is shown to be a mixture of antibiotics. The main components have been named rubiflavin A (**3**) and rubiflavin B (**2**) and are identified by NMR. spectroscopy as desacetylpluramycin A and kidamycin, respectively.

The antitumor antibiotic 'rubiflavin'[3]) was isolated from a *Streptomyces* species and characterized by *Aszalos et al.* in 1964 [1]. Purification by countercurrent distribution led to a sample which was thought to be uniform. Combustion analysis suggested the molecular formula $C_{23}H_{29-31}NO_5$, titration gave an equivalent weight of 386–430, while ultracentrifugation indicated a molecular weight of 412 [1]. No other effort has been undertaken after those early studies to clarify the structure of 'rubiflavin'. On the other hand, the antibiotic was intensely used in biological and biochemical investigations, since it was found to bind to DNA [2] [3] and inhibit its synthesis [3] [4].

The spectral data published by *Aszalos et al.* [1] closely resembled those of hedamycin (**1**), kidamycin (**2**) and related compounds [5]; thus, 'rubiflavin' seemed to belong to the pluramycin group of antibiotics. This prompted us to investigate its nature. A ^{13}C-NMR. spectrum of the crude 'rubiflavin' obtained from *Aszalos et al.* further indicated its close relation to the pluramycin antibiotics. The spectrum displayed all the resonances of the two tetrahydropyran rings E and F as in the corresponding spectra of hedamycin (**1**) and kidamycin (**2**) [6]. These data show that rings E and F are not acetylated in 'rubiflavin', which is consistent with the IR. spectrum of the antibiotic, where no ester carbonyl absorption can be discerned. The ^{13}C-NMR. signals of 'rubiflavin' in the region of sp^2-hybridized C-atoms also corresponded more or less to those usually observed in pluramycin antibiotics [6]. However, here as well as in the methyl region, signal clusters were observed, indicating that 'rubiflavin' was a mixture, the components of which seemed to differ only in the side chain at C(2).

[1]) Part of the planned dissertation of *H. N.*

[2]) A full paper will be published later.

[3]) 'Rubiflavin' – in quotation marks – will be used in this paper to denote the samples described by or obtained from *Aszalos et al.* [1].

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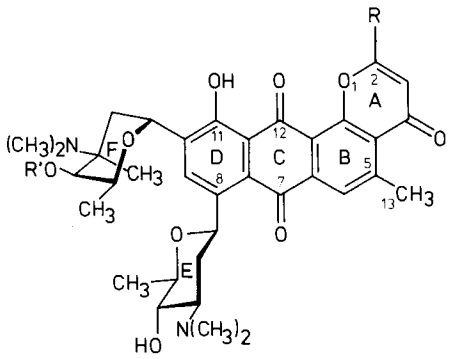
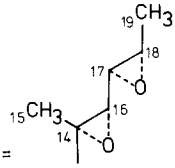
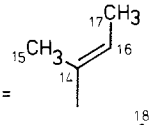
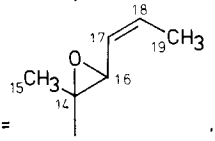
Scheme[4])



1 hedamycin R =  , R' = H

2 kidamycin (rubiflavin B) R =  , R' = H

3 rubiflavin A R =  , R' = H

4 pluramycin A R = // , R' = Ac

Attempts to isolate pure components by preparative thin layer or column chromatography using a wide variety of solvent systems and adsorbents remained unsuccessful. Antibiotic mixtures in the pluramycin series are known to be very hard to separate (*cf. e.g.* the reports on indomycins [7] and griseorubins [8]). HPLC. on silica gel with dichloromethane/conc. ammonia 99.55:0.45 containing increasing amounts of methanol (3→15%) as the mobile phase then proved to

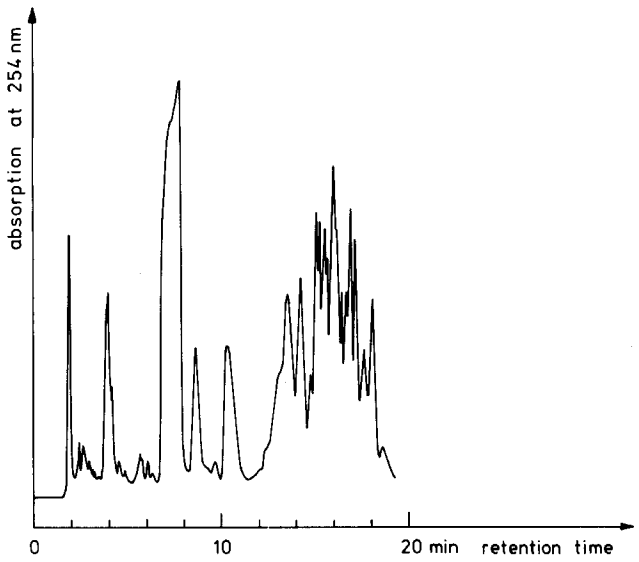


Fig. 1. *HPLC. of crude 'rubiflavin'*

[4]) The configurations of the side chains at C(2) relative to the tetrahydropyran rings E and F have not been determined in compounds **3** and **4**.

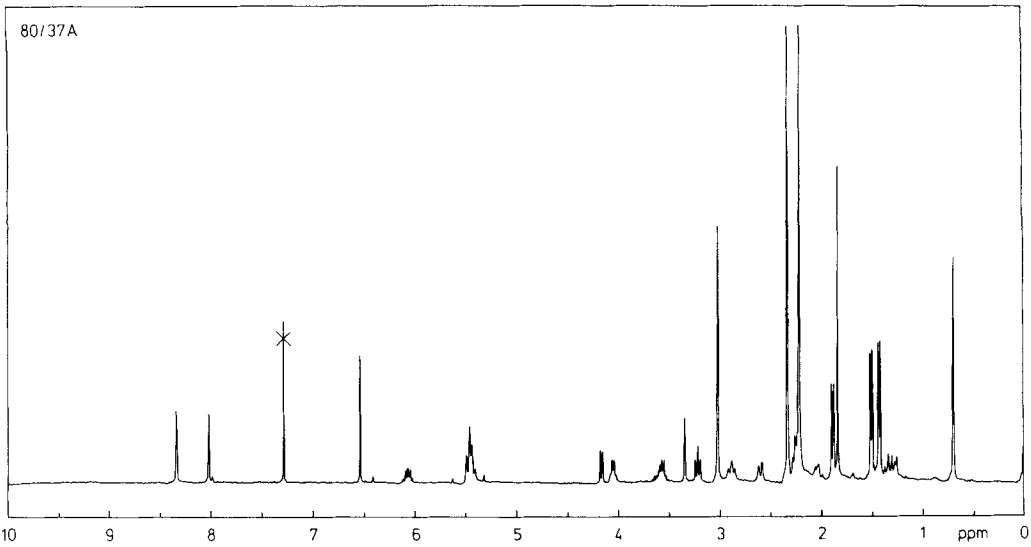


Fig. 2. *360-MHz-^{1}H-NMR. spectrum of rubiflavin A in $CDCl_3$;* the phenol resonance (14.1 ppm) is not shown.

be more successful. The chromatogram revealed that crude 'rubiflavin' was indeed a very complex mixture (see *Fig. 1*). However, some of the components might be artifacts formed during isolation or storage, as pluramycin antibiotics tend to decompose in solution or upon contact with acids or bases. The substance causing the largest peak in the chromatogram and accounting for *ca.* 20% of the crude material was collected using preparative HPLC. ^{1}H-NMR. spectroscopy revealed that this sample was still a mixture of several compounds. Further separation could be achieved by ion-pair HPLC. on a reversed phase column [9]. Three compounds could be isolated as amorphous solids and were named in the order of increasing retention times as rubiflavins A, B and C, respectively.

Rubiflavin A (*ca.* 6% of the crude 'rubiflavin') has a 360-MHz-^{1}H-NMR. spectrum that exhibits all the well known signals of the aromatic nucleus and the tetrahydropyran rings E and F (see *Fig. 2*). The remaining resonances belong to the side chain at C(2) and indicate that it contains a CH_3–CH=CH and possibly a trisubstituted epoxide group. Extensive homonuclear decoupling experiments confirmed the assignments and showed the double bond to have the (*Z*)-configuration as revealed by the 10.5 Hz coupling of the olefinic protons. All the structural features derived so far were fully corroborated by the 90.5-MHz-^{13}C-NMR. spectrum (see *Fig. 3*). All the resonances are virtually the same as in hedamycin (**1**) except those of the side chain C-atoms (labeled with * in *Fig. 3*). The lines at 59.1 and 61.6 ppm belong to the epoxide moiety (C(14) and C(16), respectively), those at 134.0 and 123.3 ppm to the olefinic C(17) and C(18), respectively. It can be seen from the chemical shifts of C(15) (14.5 ppm) and C(19) (13.8 ppm) that both these side chain methyl groups experience an upfield shift which must be due to a synperiplanar γ-group (*cf.* [10]). This confirms the (*Z*)-configuration at the

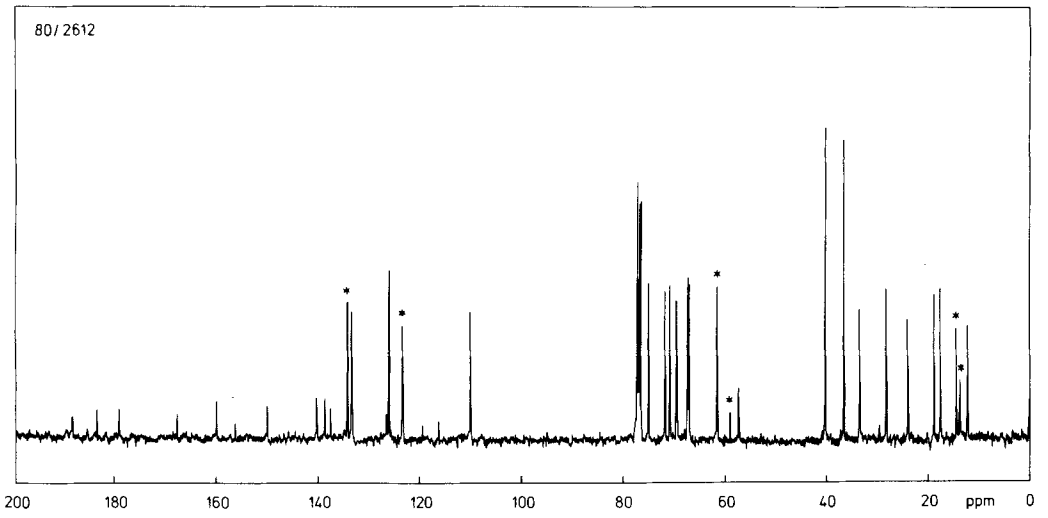


Fig. 3. *90.5 MHz-^{13}C-NMR. spectrum of rubiflavin A in $CDCl_3$;* asterisks denote resonances of side chain C-atoms.

double bond derived from the ^{1}H-NMR. data, and proves the epoxide configuration shown in formula **3**. Further support of these configurations came from NOE difference spectra. Irradiation of $H_3C(15)$ gave a distinct enhancement of the H–C(17) resonance, while irradiation of $H_3C(19)$ led to an NOE for H–C(16). Thus, rubiflavin A (**3**) is the hitherto unknown desacetyl derivative of pluramycin A (**4**) [10] [11], its molecular formula and molecular weight being $C_{41}H_{50}N_2O_{10}$ and 730.86, respectively.

Rubiflavin B (*ca.* 7% of the crude 'rubiflavin') was identified as kidamycin (**2**). The ^{1}H-NMR. spectra and HPLC. retention times of the two samples were identical.

Rubiflavin C still seems to be a mixture according to the 360-MHz-^{1}H-NMR. spectrum. The side chains of the components are possibly dienes similar to those in the indomycinones [7] [12].

The consequence of our findings, which have shown the major constituents of 'rubiflavin' to have molecular weights that are roughly double what *Aszalos et al.* have determined, is that some of the conclusions made regarding 'rubiflavin'-DNA interactions should be reconsidered (*cf. e.g.* [3]).

Finally, it is noteworthy that again pluramycin and kidamycin derivatives have been found to be produced by the same microorganism; pluramycin A and neopluramycin (3″-*O*-acetylkidamycin) [11] constitute a similar case.

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