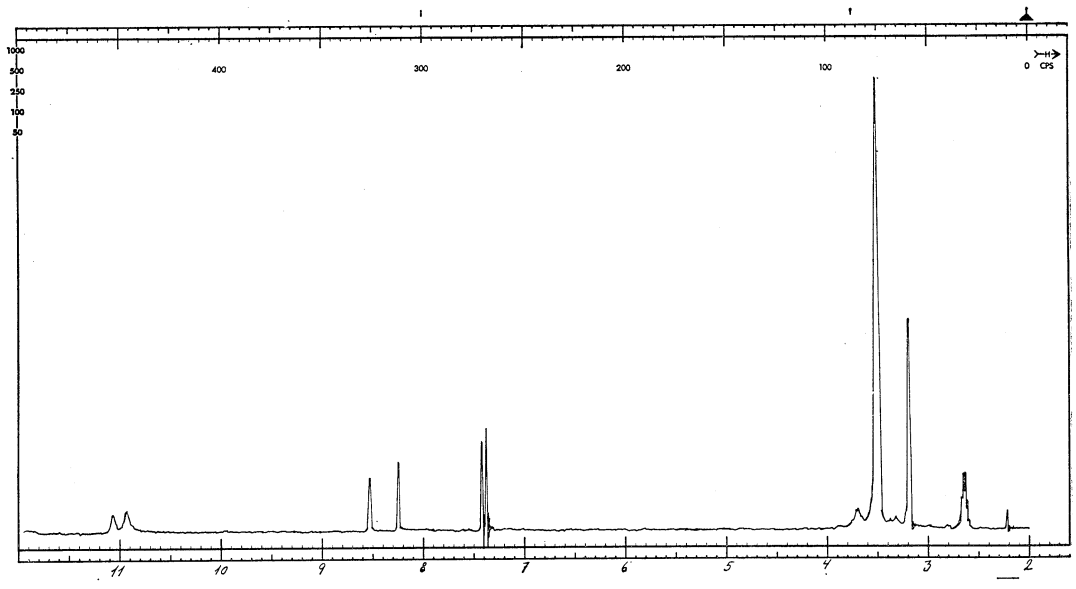
NEW ANTIBIOTICS CONTAINING THE 1,2-DITHIOLO[4,3-b]PYRROLE RING SYSTEM

Sir :

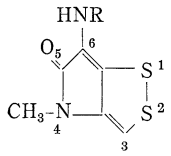
In the course of our search for new antibiotics from actinomycetes it was found that the culture fluid of an unidentified *Streptomyces* species, isolated from a soil sample collected near Copenhagen, showed strong activity against *Neisseria* and certain other gram-negative organisms. The active principle was extracted from the clarified fermentation broth at pH 2 with ethyl acetate, and the concentrated extract, which according to bioautography of thin-layer chromatograms* on agar plates inoculated with *Neisseria gonorrhoeae* contained at least two antibiotically active compounds, was subjected to a 32 tube countercurrent distribution in the system ethyl acetate - water (pH 2) to separate the components. The main component, designated vD 844, appeared in tubes 15~20, and crystallized from ethyl acetate as neutral, optically inactive, yellow prisms, m. p. 181~182°C. Its elementary analysis and mass-spectrum (peak at m/e 214 (M^+)), are consistent with the formula $C_7H_6N_2O_2S_2$. The UV-spectrum (EtOH) shows absorption maxima at 231 mμ (ε 6600) and 367 mμ (ε 15800), and the IR-spectrum (KBr) contains bands at 3380, 3080, 2960, 2740, 1675 (sh), 1665, 1572, 1349, 1308, 1229, 1102, 818, 782 and 706 cm^{-1}.

The melting point as well as the UV- and IR-spectra of vD 844 are nearly identical with those reported for the antibiotics thioaurin[1] and HA-9[2]. No structures have been assigned to these presumably identical antibiotics, but the composition and spectral data of vD 844 suggest a close relationship to the group of antibiotics containing the 1,2-dithiolo [4,3-b] pyrrole ring system, which include thiolutin (**Ia**)[3], aureothricin (**Ib**)[3], isobutyropyrrothin (**Ic**)[4], and holomycin (**IIb**)[5]. Treatment of vD 844 with boiling 6 N hydrochloric acid for two minutes afforded a crystalline hydrochloride $C_6H_7ClN_2OS_2$, m. p. 210~215°C, showing UV-maxima (EtOH) at 231 mμ (ε 5950), 298 mμ (ε 5950) and 391 mμ (ε 12950). This compound is non-identical with the isomeric hydrochloride of pyrrothin (**Id**)[3], and its acetylation product $C_8H_8N_2O_2S_2$, m. p. 195~196°C, which exhibits UV-maxima (EtOH) at 230 mμ (ε 5300) and 362 mμ (ε 17800), differs sharply from thiolutin (**Ia**)[3].

Fig. 1. 100 MHz n. m. r. Spectrum of vD 844 in DMSO-D6 at 32°C

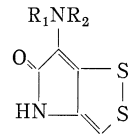


* For TLC and PLC silica gel HF_{254} was used as adsorbent. Solvent system : Methylene chloride-methanol (9 : 1).



I

a : R=$COCH_3$
b : R=$COCH_2CH_3$
c : R=$COCH(CH_3)_2$
d : R=H



II

a : R_1=H, R_2=CHO
b : R_1=H, R_2=$COCH_3$
c : R_1=R_2=H
d : R_1=CH_3, R_2=CHO
e : R_1=CH_3, R_2=$COCH_3$
f : R_1=CH_3, R_2=H

These findings suggested that vD 844 is best represented by formula **IId**, but because of the limited amounts available further chemical studies to confirm this view could not be performed. Through the courtesy of Dr. BIRTHE JENSEN the compound was therefore subjected to an X-ray crystallographic analysis which rigorously established the structure as **IId**[6]. The deformylated product and its acetate have consequently the structures **IIf** and **IIe**, respectively.

The n.m.r. spectrum of **IId** (cf. Fig. 1) deserves a special comment. The fact that all of the protons at ordinary temperature give rise to two signals, the spacing of which is proportional to the field strength implies that the molecule in DMSO solution exists in two forms. The relative intensity of the lines shows that the form in which the methyl protons resonate at the lowest field is slightly predominating (appr. 6 : 4).

Table 1. Chemical shift and coupling constants for methyl group and formyl protons*

Compound	δ_{CH_3}	δ_{CHO}	$J_{CH_3\text{-}H}$ MHz
DMF	2.92 (*cis*) 2.77 (*trans*)	7.95	<0.5 (*cis*) 0.7 (*trans*)
3	3.42	8.13	<0.4
4	3.10	8.43	0.8

* The spectra were measured at 32°C with a Varian HA-100 (100 MHz) spectrometer in DMSO-D6. TMS was used as internal reference.

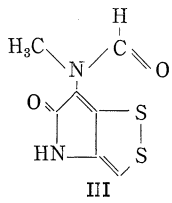
The presence of two forms can be explained by the partial double-bond character of the C-N bond in the formylamino group (cf. formulae **III** and **IV**) which makes the barrier for rotation about this bond sufficient high that separate signals can be observed for the two isomers[7,8,9] or by restricted rotation around the nitrogen-heterocycle bond. In both cases one would expect to find small couplings between the methyl group and the formyl protons. However, in the first case the coupling constants should

Table 2. Antimicrobial spectra of compounds **IIa**, **IId**, **IIe** and **IIf**

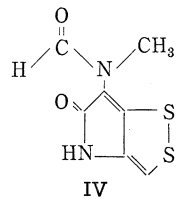
Organism	Medium	IC_{50} (μg/ml)			
		IId	**IIf**	**IIa**	**IIe**
Pseudomonas aeruginosa	1	16	40	25	16
Staphylococcus aureus ATCC 6538P	1	13	16	1.6	16
Staphylococcus aureus ATCC 6538P	3	0.50*			
Neisseria gonorrhoeae (7 strains)	3	0.011～0.040	5.0～6.3	0.008～0.080	0.063～0.160
Neisseria meningitidis (2 strains)	3	0.024～0.033	5.0～6.3	0.050	0.08 ～0.20
Neisseria catarrhalis NCTC 3622	3	0.032	5.0	0.063	0.13
Streptococcus pyogenes NCTC 6175	2	2.5			
Streptococcus faecalis ATCC 8043	1	16			
Escherichia coli	1	16	50	3.2	16
Klebsiella pneumoniae	1	10	16	0.5	16
Proteus vulgaris NCTC 4174A	1	5.6	16	0.2	16
Salmonella typhimurium NCTC 5710	1	14	32	1.6	16
Salmonella typhosa NCTC 5760	1	10	25	1.6	16
Shigella dysenteriae NCTC 8217	1	10	16	1.3	16
Haemophilus influenzae NCTC 6489	3	0.035	13	0.063	0.20
Bordetella pertussis	3	0.053	5.0	0.050	0.16
Bacillus subtilis	1	13	20	1.6	16

Media : 1. N.I.H. broth (Difco). 2. N.I.H. broth with 2.5% horse serum. 3. Blood-ascites agar. Inoculum 10^4 organisms per ml.

* It has been demonstrated that the decreased IC_{50}-value in this medium is due to the presence of hemoglobin and that a similar effect can be obtained by adding hemine or ferri ions to medium 1.



III



IV

be different in the two forms like in dimethylformamide[7] whereas in the second case the coupling would be expected to be almost unaffected by the rotation. By use of the double irradiation technique it could be shown that the observed splitting of the methyl group signals actually is due to coupling to the formyl protons and since the magnitude of the coupling constants is very similar to those in DMF (cf. Table 1) we can conclude that the spectrum can be rationalized by assuming an equilibrium between **III** and **IV**. In agreement with this interpretation is the fact that the doublet due to the CH-3 proton in **IId** collapses into a singlet at 85°±5°C. At this temperature the signals due to the formyl protons and the methyl groups have broadened considerably, and the line separations are reduced. The barrier of rotation calculated on the basis of these data is in close agreement with that recently reported for DMF[7]. The assignments used in Table 1 are based upon the close analogy to DMF for which it has been shown that the methyl group resonating at the lowest field is *cis* to the formyl proton[10].

A second, less polar, antibiotically active compound, that appeared in tubes 21~30 from the counter-current distribution, was designated vD 846. According to TLC it was contaminated with **IId** but could be purified by PLC on silica gel. It crystallized from acetone as neutral, orange prisms, $C_6H_4N_2O_2S_2$ (mass-spectrum : peak at m/e 200 (M^+)), which decomposed at 270~280°C without melting. The UV-spectrum (EtOH) shows absorption maxima at 247 mμ (ε 5800), 301 mμ (ε 3350), and 387 mμ (ε 11000), and the IR-spectrum (KBr) contains bands at 3205, 3115, 2970, 2740, 1688 (sh), 1680, 1645, 1600, 1540, 1379, 1350, 1280, 1230, 1140, 1047, 793, 748, and 707 cm^{-1}.

On basis of these data the most likely structure of vD 846 is **IIa**. This was verified by comparison with a sample of this compound prepared in an unambiguous way from holomycin (**IIb**)* : Treatment of **IIb** with boiling 6 N hydrochloric acid for two minutes gave the hydrochloride of holothin (**IIc**) which on formylation with formic acid afforded **IIa**. The product thus obtained was identical in every respect (IR, UV, TLC *etc.*) with vD 846.

Compounds **IIa**, **IId**, **IIe** and **IIf** were tested against a number of microorganisms by the serial dilution method. From Table 2, in which the 50 % inhibitory concentrations are given, it will be seen that the most striking properties of **IIa** and **IId** are their high activities against *Neisseria*, *Haemophilus influenzae* and *Bordetella pertussis*.

The acute intravenous LD_{50} in mice was determined for compounds **IIa**, **IId** and **IIf** to approximately 5~10 mg/kg. All the compounds are strongly local irritating, in particular **IIf**, which administered subcutaneously to mice in doses less than 100 μg/kg causes heavy pains.

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References

1) BOLHOFER, W. L.; R. A. MACHLOWITZ & J. CHARNEY : Thioaurin, a new crystalline antibiotic. Antibiot. & Chemoth. 3 : 382~384, 1953
2) EISENMAN, W.; P. P. MINIERI, A. ABBEY, J. CHARLEBOIS, M. MONCRIEFF-YEATES & N. E. RIGLER : A new sulfur-containing antibiotic, produced by a *Streptomyces*, active against bacteria and fungi. Antibiot. & Chemoth. 3 : 385~392, 1953
3) CELMER, W. D. & I. A. SOLOMONS : The structures of thiolutin and aureothricin, antibiotics containing a unique pyrrolinonodithiole

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nucleus. J. Am. Chem. Soc. 77 : 2861～2865, 1955

4) Bhate, D. S.; R. K. Hulyalkar & S. K. Menon : The isolation of *iso*-butyropyrrothine along with thiolutin and aureothricin from a *Streptomyces* sp. Experientia 16 : 504～505, 1960

5) Ettlinger, L.; E. Gäumann, R. Hütter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog & H. Zähner : Stoffwechselprodukte von Actinomyceten, Holomycin. Helv. Chim. Acta 42 : 563～569, 1959

6) Jensen, B. : On the crystal structure of 5-oxo-6-N-methylformylamino-4, 5-dihydro-1, 2-dithiolo[4, 3-b]pyrrole. J. Antibiotics 22 : 231～232, 1969

7) Conti, F. & W. von Philipsborn : Die Bestimmung der Energiebarriere für die gehinderte interne Rotation in N, N-Dimethylformamide durch Protonenresonanz-Spektroskopie. Helv. Chim. Acta 50 : 603～607, 1967

8) Neuman, R. C., Jr. & V. Jonas : Internal rotation in N, N-dimethylacetamide-d_3 and a structure-reactivity correlation for the rotation reaction of amides. J. Am. Chem. Soc. 90 : 1970～1975, 1968

9) Pines, A. & M. Rabinovitz : A nuclear magnetic resonance total line-shape treatment of internal rotation in dimethylformamide. Tetrahedron Letters 1968-31 : 3529～3532, 1968

10) Hatton, J. V. & R. E. Richards : Solvent effects in N.M.R. spectra of amide solutions. Mol. Phys. 5 : 139～152, 1962