C'est une originalité de ce groupe, que de permettre à des biologistes de tous les horizons de se rencontrer, et de discuter ensemble de leurs travaux non pas seulement pour confronter les résultats eux-mêmes mais peut-être surtout pour comparer les protocoles d'expériences et les méthodes d'interprétation des résultats. Ce groupe se préoccupe aussi de la documentation (colloques, bibliographie, adresses). Il s'ajoute à diverses organisations aux méthodes d'action différentes (Society for Biological Rhythm, International Society of Biometeorology, International Institute for Interdisciplinary Cycle Research, International Society for the Study of Time etc.).

Summary. Living beings show many examples of rhythmic behaviour. For the majority of biologists these are disturbing phenomena, which oblige them to think that 2 measurements taken at different times are not comparable. But these rhythms may be studied in themselves.

For several decades, biologists have demonstrated that in the functioning of an organism not only the questions what? and where? have to be answered but also the question when? A striking example is the discovery of photoperiodism.

The study of the relations between living beings and time is at present approached in several very different ways. BÜNNING, working mainly on plants, has underlined the internal determination of many rhythms, has discovered the participation of endogenous rhythms in photoperiodism and has described the general rules of the course of the endogenous rhythms, so as to allow an experimental approach in the research of the responsible mechanisms. It is this direction of research which is discussed here, while comparing it to the methods of study of other chronobiological schools, in particular to those which especially use animals or even man as material, those which concern more directly research on biochemical mechanisms and those which put forward the practical applications.

The laws of circadian rhythms are summarized: frequent self-maintenance in free-running, a persistent period of about 24 h not very sensible to temperature, possibility of being regulated by external stimulations etc.

The author shows that rapid rhythms (at least concerning plants) obey analogous laws but with slight variations: spontaneous or provoked induction by only one stimulus, self-maintenance in free-running but less regulation by periodical stimulations of a slightly different length and stronger dependence of the persistent period on temperature.

The low frequency (annual, etc.) rhythms behave comparably.

So the laws of rhythms established by BÜNNING, mainly for circadian rhythms, seem to concern the biological rhythms as a whole.

SPECIALIA

Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Ответственность за короткие сообщения несёт исключительно автор. – El responsable de los informes reducidos, está el autor.

The Total Structure of Viomycin, a Tuberculostatic Peptide Antibiotic

Viomycin was independently isolated in 1951 by two laboratories[1,2] from the actinomycetes designated as *Streptomyces puniceus* and *Streptomyces floridae*. It has since been isolated from a number of other *Streptomyces* species and is identical with vinactin A[3] from *S. vinaceus*. The possibility that viomycin belongs to a closely related family of antibiotics is exemplified by the similarity of its physical, chemical and pharmacological properties to those of the capreomycins[4], from *S. capreolus* and tuberactinomycin[5] from *S. griseoverticillatus*.

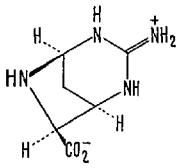
The antimicrobial activity of viomycin is restricted to *Mycobacterium tuberculosis* and it has found limited clinical use for tubercular patients who have failed to respond to more classical chemotherapy.

Initial chemical investigations established that the antibiotic affords crystalline sulphate, reineckate, picrate, and hydrochloride derivatives[6]. Our own analytical data on these derivatives, together with molecular weight determinations by a variety of methods, leads to a molecular formula $C_{25}H_{43}N_{13}O_{10}$ (M.W. 689). This differs appreciably from earlier assignments[6-8] but is completely in accord with our proposed structure (VII) (see below). It is also supported by a recent X-ray crystallographic molecular weight determination which gives a value of 1025 for viomycin sulphate picrate, corresponding to a molecular weight of 686 for the free base[9]. Spectroscopically, viomycin is characterized by a strong UV-absorption at 268 nm. (ε, 24,000) in neutral and acidic media which shifts to 285 nm. (ε, 15,000) in 0.1 *N* sodium hydroxide[2]. The NMR-spectrum (D_2O) of the antibiotic exhibits a low field signal (singlet) at 2τ corresponding to one proton[8].

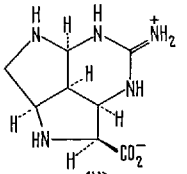
The antibiotic is a strong base which gives positive Sakaguchi, Fehling, ninhydrin and biuret tests[2]. Total acid hydrolysis affords the amino-acids, L-serine, L-α-β-diaminopropionic acid, L-β-lysine and viomycidine (ratio 2:1:1:1 respectively) as well as one equivalent of urea and varying amounts of carbon dioxide and ammonia.

Early in our work we observed that acid hydrolysis consistently yielded trace amounts of glycine and a further basic amino-acid, viocidic acid.

The amino-acid viomycidine, after some initial controversy, was assigned structure (I)[10] and this has recently been confirmed by several X-ray crystallographic analyses[11–13]. However, although our own investigation supported the structure (I) for viomycidine, further extensive degradative evidence, in particular base hydrolysis which yielded glycine and 2-aminopyrimidine, and an X-ray crystallographic structure determination on viocidic acid (II)[14], clearly demonstrated that viomycidine was not present as such in the intact antibiotic.



(I)



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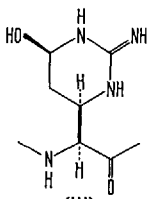

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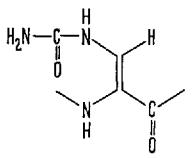

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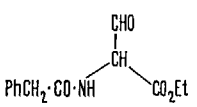

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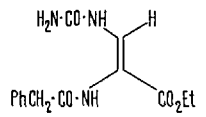
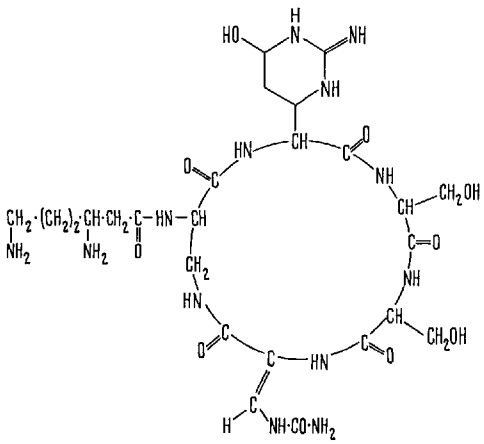


(VI)



(VII)

This led us to suggest[14,15] that the structural unit (III) is present in viomycin and several transformations of viomycin were interpreted on this basis, all of which parallelled closely those of the analogous fragment which exists in the molecule of the fish poison tetrodotoxin[16]. Other workers have recently presented supporting evidence for the existence of the guanidine-carbinol unit in viomycin[13,17].

A further complicating feature of the structure of viomycin is the unit responsible for the ultraviolet chromophore, especially as this fragment cannot be isolated intact on total hydrolysis. The lability of both the chromophoric and the guanidine-carbinol units has presented considerable difficulties in interpreting degradative results. Earlier formulations of the chromophore combined both these units[7,8], whereas 2 recent publications have misinterpreted results relating to the guanidine unit and have taken no account of the chromophore[18,19]. We observed that when viomycin is hydrolyzed with 0.1 *N* hydrochloric acid, one equivalent of urea is liberated with concomitant disappearance of the UV-absorption and the low field proton[20]. The resultant desureaviomycin gives a deep red ferric test as well as readily reducing Tollman's reagent, silver nitrate, and copper acetate. Although lacking UV-absorption in acidic media, it shows a strong maximum at 272 nm in basic solution.

A key observation in our investigation has been that desureaviomycin can be smoothly reconverted to viomycin in the presence of excess urea and dilute acid[21]. This observation not only established that no deep-seated rearrangement had occurred in the course of the hydrolysis, but also provided an extremely facile route to a series of modified viomycin derivatives containing substituted ureas. This evidence, combined with degradative and spectral data, led us to suggest that the ultraviolet chromophore of viomycin is due to the presence of the dehydroserine ureide (IV) which was also the source of the small amounts of glycine formed on acid hydrolysis of viomycin. The close similarity in chemical, physical, and spectral properties of the penaldate (V)

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and its ureide (VI) with those of desureaviomycin and viomycin respectively has provided strong support for this proposal[22].

The sequence of the amino-acids in viomycin has been determined from the evidence of end group analyses of viomycin itself and of the structure of the dipeptides obtained from partial base hydrolysis. The molecular structure is shown in formula (VII) and the assignment is in accord with the physical and spectral properties of the antibiotic.

Investigations on the capreomycin complex are still in progress and will be reported at a later date, although all the components have been shown to contain the chromophoric system (IV). The units (III) and (IV) are derived from dehydroarginine and dehydroserine respectively and the related biosynthesis of the dehydroamino-acid units and D-amino acid units frequently found in microbial peptides has been discussed elsewhere[23].

Zusammenfassung. Die Struktur von Viomycin wird durch seine Zerfallseigenschaften bestimmt und das Vorhandensein von Guanidin-Carbinol sowie von Dehydroserin oder Formylglycin bestätigt.

B. W. Bycroft[24], D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson[24,25] and T. Webb

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The Influence of the Second Heteroatom on the Spectra of 3-Alkyl-2-Acylmethylenbenzazolines

The influence of the second heteroatom on the chemical properties[1], IR-[2], electronic[3-6] and NMR[7]-spectra of the benzazoles have been already investigated. The differences observed have been interpreted in terms of the electron-releasing mesomeric effect of these atoms. For instance, the fact that the benzthiazoles absorb at higher wavelengths than the corresponding benzoxazoles has been explained either by the larger +M effect of the sulfur atom in the excited state[3-5] or by its ability to be a conjugation transmitter[6].

In contrast with these findings, our measurements on 3-alkyl-2-acylmethylenebenzazolines (I) led us to the conclusion that the influence exerted by the heteroatom X on the electronic- and IR-spectra may be correlated with the inductive effect of X. This effect outweighing the mesomeric one, controls the phenomena observed. Thus, the increasing order of the frequency of the λ_{max} absorptions (Table I) and of $\nu_{C=C}$ and $\nu_{C=O}$ (Table II), respectively, is as expected, taking into account the inductive effect of the heteroatom X, namely: Se < S < N < O.

Table I. λ_{max} Absorption bands[a] of the compounds I

R	X[b] O (3.5) λ_{max} nm (ν cm^{-1})	ε	N–CH$_3$ (3.0)[c] λ_{max} nm (ν cm^{-1})	S (2.5) λ_{max} nm (ν cm^{-1})	ε	Se (2.4) λ_{max} nm (ν cm^{-1})	ε
H[a]	357.1 (28,000)	39,700	368 (27,173)	381 (26,247)	37,200	382.2 (26,164)	37,900
4′-NO$_2$[a]	386 (25,906)	18,600	401 (24,937)	411 (24,330)	28,300	414 (24,154)	31,300
3′,5′(NO$_2$)$_2$[b]	384.6 (26,000)	18,200	–	411.2 (24,319)	21,900	413.9 (24,160)	21,000

[a] Measurements made in: a) methanol; b) DMF. [b] Pauling's[8] electronegativities scale. [c] The optical density varies with time.

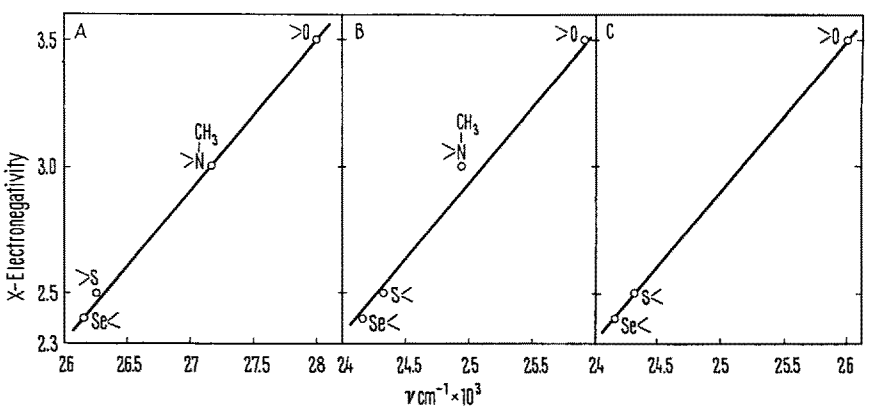

Fig. 1. The relation between the frequencies of λ_{max} absorption bands and the electronegativity of X in compounds I: A) R = H; B) R = 4′-NO$_2$ and C) R = 3′m5′(NO$_2$)$_2$.