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DIFFERENCE OF VITAMYCIN A FROM OTHER NATURAL ANALOGS OF PRODIGIOSIN

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In 1964, one of us [1] described the production and some properties of vitamycin A, the main component of the biostimulator vitamycin formed by Actinomyces aureoverticillatus. It was established that this compound is similar in many particulars to prodigiosin [1] and prodigiosin-like pigments produced by some actinomycetes and bacteria.

The data which we then obtained on the chemical and physicochemical properties of vitamycin A showed that it is distinguished from other reported substances of similar type and permitted a preliminary structural formula to be proposed for it. In 1966, new papers appeared on natural pigments very close to vitamycin A; on the one hand Harashima, Tsuchida, and Nagatsu [2] reported the isolation and some properties of "prodigiosin-25C," and on the other hand, Wasserman et al. reported the structural formulas of two analogs of prodigiosin [3, 4]. In view of this, it was necessary to publish our materials showing the difference of the substance that we isolated from those described by the other workers.

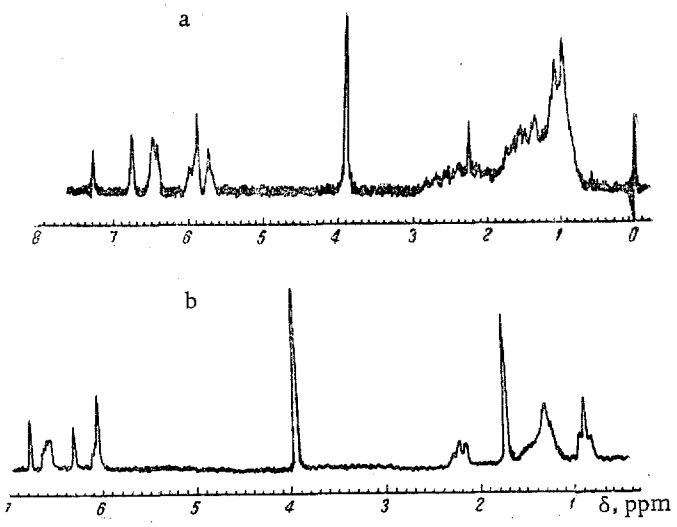
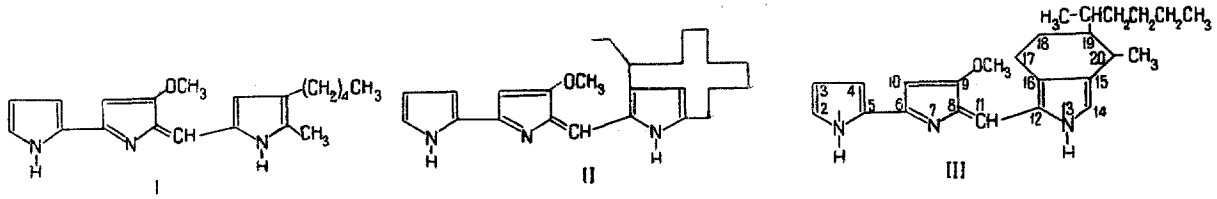


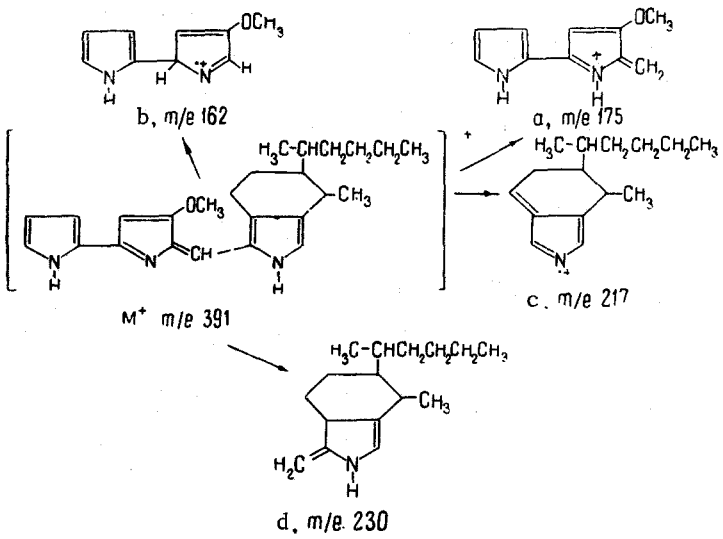
Fig. 1. NMR spectrum of vitamycin A (a), (taken in $CDCl_3$ on a JNMC-60 instrument) and of prodigiosin (b), taken in $CDCl_3$ on a JNM4-H-100 instrument).

A mass spectrometric determination of the molecular weight of vitamycin A (391) showed that its empirical formula corresponds to $C_{25}H_{33}N_3O$; this is in satisfactory agreement with the results of elementary analysis. Consequently, this compound differs from undecylprodigiosin, which is identical with prodigiosin-25C and from the previously described homolog of prodigiosin [5].

The properties of vitamycin A do not agree with those established by Wasserman and Rodgers for the analog of prodigiosin $C_{25}H_{33}N_3O$ with the structural formula (II). Thus, judging from the NMR spectrum (Fig. 1, a) and the mass spectrum (Fig. 2, a), vitamycin A has three C-methyl groups while in formula (II), there is only one. Vitamycin A contains (judging from the NMR spectrum) two hydrogens in the α-positions of pyrrole rings, while in formula (II) there is only one hydrogen in the α-position of a monosubstituted pyrrole ring. There are other noncorrespondences between formula (II) and the properties of the substance that we have studied. All this permits the conclusion that vitamycin A is different from all the known analogs of prodigiosin and is a new compound. The results that we have obtained permit us to propose for it the provisional structural formula (III), which can be substantiated by the following:



The conclusion of the presence in vitamycin A of a monosubstituted pyrrole ring and a second pyrrole ring containing a methoxy group in the β-position follows from data on its oxidation with potassium permanganate and chromic anhydride, under which conditions it is possible to isolate the same products (pyrrole carboxylic acid, maleimide, and methoxymaleimide), as in the oxidation of prodigiosin under similar conditions. The great similarity of the NMR spectra of vitamycin A and prodigiosin (see Fig. 1) in the region from 3 to 12 ppm indicates the identity of the bipyrryl moieties of the two compounds. This conclusion is also in harmony with the presence in the mass spectra (see Fig. 2) of those compounds of well-defined peaks with m/e 175 (ion *a*) and 162 (ion b) the mass numbers of which are shifted by one unit in the case of the deuterated analogs of prodigiosin and vitamycin A (to m/e 176 and 163, respectively). The formation of ions *a* and b can be represented by scheme 1:



The structure of the third pyrrole ring differs markedly from the corresponding moiety of prodigiosin. A consideration of the NMR spectra in the 5.5–7.0 ppm region permits the conclusion that the singlet at 6.75 ppm corresponds to the proton of the methene group at C_{11}, which is confirmed by the NMR spectra of bipyrrylylpyrrylmethenes, samples of which were kindly given to us by Prof. Johnson (Nottingham University). The signal at 6.4–6.5 ppm is due to the protons at C_2 and C_4 of the monosubstituted pyrrole nucleus, while the peaks at 6.1 and 5.88 ppm, respectively, are due to the two protons in the β-positions of the first and second pyrrole nuclei (at C_3 and C_{10}). Thus, only the one proton present in the third pyrrole ring has a very different nature in the two compounds concerned (6.35 ppm in prodigiosin and 5.72 ppm in vitamycin A).

In the presence of a small amount of trifluoroacetic acid, the NMR spectrum of vitamycin A in $CDCl_3$ undergoes a change; there is a shift to weaker fields of the signals at 6.4 ppm and at 5.72 ppm by approximately 0.1 ppm, while the signal at 6.1 ppm (due to the protons in the β-positions of the first and second pyrrole nuclei) does not change appreciably. This permits the conclusion that the signal at 5.72 ppm is due to the hydrogen in the α-position of the third pyrrole nucleus (at C_{14}). It follows from this that the latter lacks hydrogen in the β-positions, and this means that there are substituents at C_{16} and C_{15}.

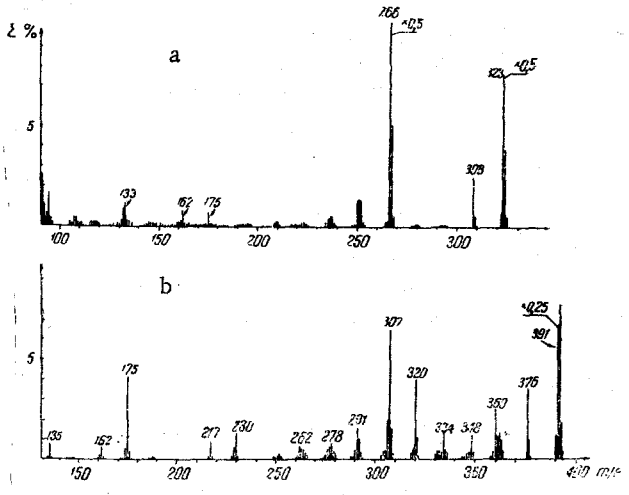
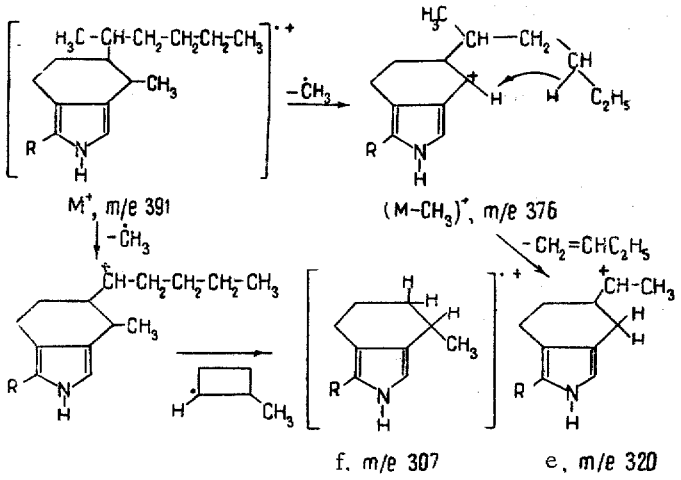


Fig. 2. Mass spectra of vitamycin A (a), (taken on an MKh-1303 instrument with direct introduction into the source, 160° C, ionization energy eV) and of prodigiosin (b), (taken on an MKh-1303 instrument with direct introduction into the source, 130° C, ionization energy 35 eV).

The intensity of the signals in the 1.7–2.8 ppm region (corresponding to three protons) shows that there is a total of three protons in positions 17 and 20 (adjacent to the heteroaromatic system). This is shown still more clearly when the NMR spectrum of vitamycin A is taken at 100 MHz in benzene; the signals under consideration are located in the 2.8–3.5 ppm region (with intensities of 1 and 2 proton units, respectively). The structure of the third pyrrole nucleus of vitamycin A is also confirmed by the presence in the mass spectrum of the latter of isolated peaks with m/e 217 (ion c) and 230 (ion d) (see scheme 1).

On the other hand, in the NMR spectrum of vitamycin A there is a well-defined peak in the 0.7–1.2 ppm region, the intensity of which corresponds to the presence of three methyl groups. In harmony with this is the presence in the mass spectrum of vitamycin A of the peak of the ion $(M-CH_3)^+$ (m/e 376). Finally, the structure of the aliphatic side chain of vitamycin A can be deduced on the basis of the following considerations. The mass spectrum of vitamycin A contains peaks of approximately equal intensities with m/e 362, 348, and 384, differing from one another by 14 mass units (CH_2 groups), and also peaks of considerable intensity with m/e 320 (the ion e) and 307 (the ion f). This permits the conclusion that the aliphatic chain of vitamycin A consists of a straight chain of five carbon atoms and one CH_3 group on the C atom adjacent to the carbocyclic part of the molecule. The ions e and f are probably formed in the following way (scheme 2):



When the NMR spectrum of vitamycin A is recorded in benzene, it can clearly be seen that the signal of the methyl group present in the carbocyclic ring adjacent to the heteroaromatic system appears in the form of a well-defined doublet (J_{CH_3CH} ~7 Hz) which confirms its presence at position 17 or 20. Consequently, the branched six-carbon chain is located in position 18 or 19.

Thus, the provisional structural formula (III) proposed for vitamycin A in which the choice between position 17 and 20 for the methyl group and between position 18 and 19 for the aliphatic chain remains to be made can be considered as satisfactorily substantiated although, of course, the indirect nature of the proof and the indeterminacy mentioned above do not permit it to be definitive.

Conclusions

1. Vitamycin A has been shown to be different from other known natural pigments analogous to prodigiosin.

2. On the basis of a study of the products of the oxidation of vitamycin A, its NMR spectra taken in various solvents, and its mass spectrum, and a comparison with the analogous data for prodigiosin and synthetic bipyrrylylpyrrylmethenes, the provisional formula (III) has been proposed for vitamycin A.

REFERENCES

1. Yu. M. Khokhlova, A. V. Puchnina, and O. I. Artamonova, Biokhim., 29, 841, 1964.
2. K. Harashima, N. Tsuchida, and J. Nagatsu, Agric. Biol. Chem., 30, 309, 1966.
3. H. H. Wasserman and G. C. Rodgers, 4-th International Symposium on the Chemistry of Natural Products, Stockholm, Abstract book, 107, 1966.
4. H. H. Wasserman, G. C. Rodgers, and D. D. Keith, Chem. Comm., 825, 1966.
5. E. Dietzel, Z. Physiol. Chem., 284, 262, 1949.
6. R. A. Nicolaus, R. Nicoletti, and F. Arcamone, Ricerca Sci., 28, 2314, 1958.

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