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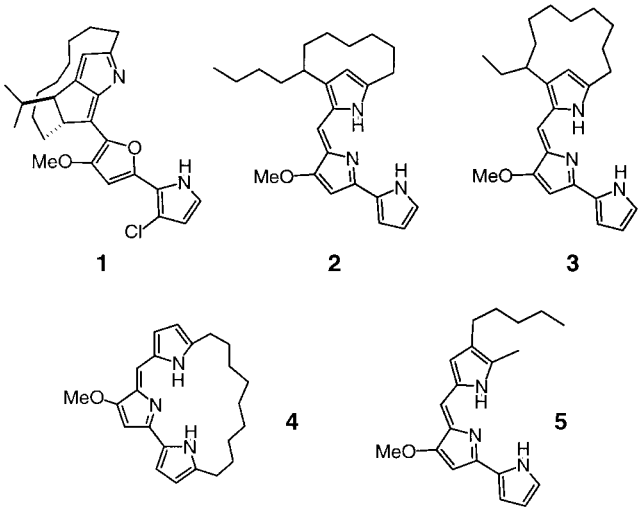

Studies on DNA Cleavage by Cytotoxic Pyrrole Alkaloids Reveal the Distinctly Different Behavior of Roseophilin and Prodigiosin Derivatives

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KEYWORDS:

alkaloids · antitumor agents · DNA cleavage · prodigiosins · pyrroles

Roseophilin (**1**), a secondary metabolite isolated from a culture broth of *Streptomyces griseoviridis*, has attracted considerable attention for the intricate molecular topology of its ansa-bridged azafulvene core as well as for the potent cytotoxicity against various human cancer cell lines.[1, 2] Several conceptually different approaches aiming at the total or partial synthesis of this challenging target have been reported and a set of functional derivatives has been prepared.[3–5] The biological mode of action of **1**, however, still remains elusive.

The closest structural relatives to roseophilin occurring in nature are the members of the prodigiosin family such as **2** – **4**.[6–8] Like **1**, these alkaloids contain an azafulvene motif as well as an ansa bridge, but incorporate a methoxypyrrole rather than a methoxyfuran as the central ring into their heterocyclic perimeter. In view of these structural similarities and the potent cytotoxic and immunosuppressive activities of compounds **2** – **5** and their congeners,[9, 10] it seems appropriate to study whether roseophilin (**1**) and the prodigiosins share a common mode of action toward biological receptors. Having access to both series by our total synthesis programs,[3, 8, 11] we are able to perform studies along these lines. An interim report comparing the ability of these compounds to damage double-stranded DNA in the presence of metal cations is summarized below.

Very recently, it has been demonstrated that prodigiosin (**5**) binds to DNA and produces oxidative strand cleavage if administered in combination with Cu^{II} salts.[12] This biological effect is triggered by the formation of π-radical cations through oxidation of the electron-rich pyrrolylpyrromethene chromophor of **5** by the metal cation and may account for the cytotoxicity of this alkaloid.[13]

By using the same assay system, we were able to gain deeper insights into the structural requirements for effective cleavage of DNA by prodigiosin derivatives. As can be seen from the agarose gel depicted in Figure 1, neither nonylprodigiosin (**4**)[8b] per se (lane 3) nor Cu^{II} alone (lane 2) damage purified double-stranded plasmid DNA of the bacteriophage ΦX174 (lane 1).[14, 15] In contrast, a combination of both is very effective: The progress of strand cleavage caused by **4**·$Cu(OAc)_2$ with increasing incubation time is depicted in lanes 4 – 13. It is clearly visible that the

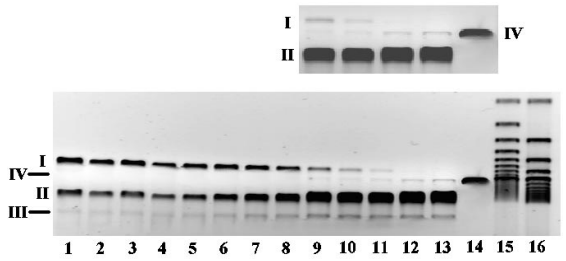

Figure 1. *Result of agarose gel electrophoresis showing the extent of DNA cleavage produced by nonylprodigiosin (**4**) in the presence of $Cu(OAc)_2$ with increasing incubation time at 37 °C. Lane 1: DNA alone; lane 2: DNA + Cu^{II}; lane 3: DNA + **4**; lanes 4 – 13: DNA + **4** + Cu^{II} after the following incubation times: 0 min (4), 5 min (5), 10 min (6), 15 min (7), 20 min (8), 30 min (9), 45 min (10), 60 min (11), 90 min (12), 120 min (13); lane 14: linear DNA formed from scDNA by using the restriction endonuclease* Xho*I; lane 15: DNA marker (500 base pairs molecular weight difference); lane 16: DNA marker (1000 base pairs molecular weight difference). The insert at the top shows the relevant detail of lanes 10 – 14 at higher magnification.*

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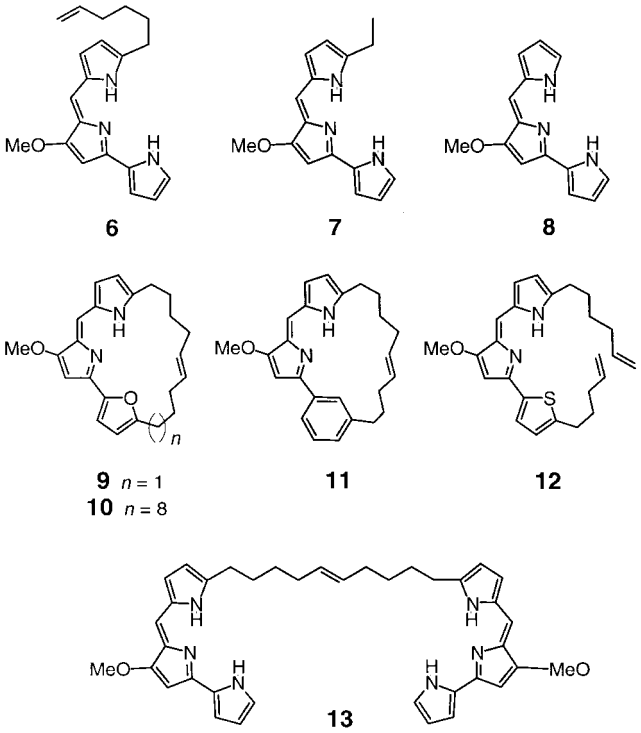
nicked form **II** as well as the concatemeric form **III** constantly gain intensity at the expense of the supercoiled (sc) form **I**. After ca. 60 min at 37 °C, the latter has almost completely disappeared, whereas band **IV** corresponding to linear DNA formed by double-strand cleavage can be detected (compare with lane 14 showing linear DNA obtained with the restriction endonuclease *Xho* I).[16]



The efficacies of a set of different prodigiosin derivatives in inducing single- and/or double-strand cleavage of supercoiled DNA in the presence of Cu^{II} are compared in Figure 2. Lanes 1 – 3 refer to control experiments showing that neither the truncated prodigiosin **6**[10, 16] nor $Cu(OAc)_2$ alone lead to any noticeable effect. In line with the results described above, a combination of both completely degrades scDNA (**I**) after 60 min by strand cleavage, since only bands of the nicked (**II**), concatemeric (**III**), and linear forms (**IV**) are visible after this incubation time.

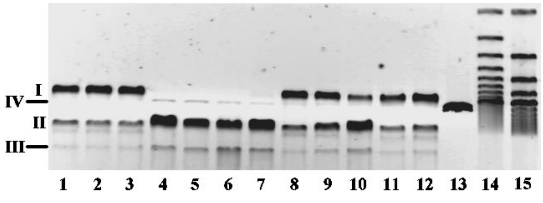
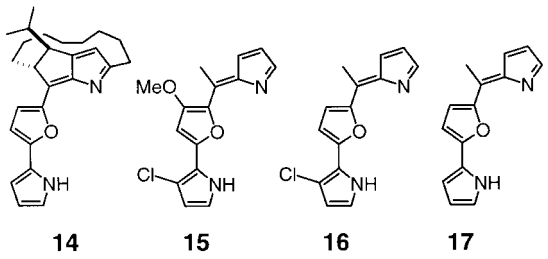


Figure 2. *Result of agarose gel electrophoresis showing the extent of DNA cleavage produced by different prodigiosin derivatives in the presence of $Cu(OAc)_2$ after an incubation time of 60 min at 37 °C. Lane 1: DNA alone; lane 2: DNA + **6**; lane 3: DNA + Cu^{II}; lane 4: DNA + **6** + Cu^{II}; lane 5: DNA + **7** + Cu^{II}; lane 6: DNA + **8** + Cu^{II}; lane 7: DNA + **4** + Cu^{II}; lane 8: DNA + **10** + Cu^{II}; lane 9: DNA + **9** + Cu^{II}; lane 10: DNA + **13** + Cu^{II}; lane 11: DNA + **11** + Cu^{II}; lane 12: DNA + **12** + Cu^{II}; lane 13: linear DNA; lane 14: DNA marker (500 base pairs molecular weight difference); lane 15: DNA marker (1000 base pairs molecular weight difference).*

Compounds **7** and **8** bearing a shorter or no alkyl side chain on the pyrrole ring C, respectively, behave similarly (lanes 5 and 6), as does nonylprodigiosin (**4**) (positive control, lane 7). Interestingly, product **13** containing two pharmacophore units is less efficient in cleaving the plasmid (lane 10). Most important with regard to structure – activity relationships, however, is the finding that all prodigiosin analogues in which one pyrrole ring has been replaced by another aromatic entity (furan, thiophene, benzene) show no appreciable activity (lanes 8, 9, 11, 12).



These close ties between the nature of the arene rings and the capacity of the compounds to cleave DNA is corroborated by the screening of roseophilin (**1**),[3a] its elaborate analogue **14**, differing from the natural product only in the substitution pattern of the heteroaromatic rings,[3c] and of the truncated chromophore models **15** – **17** (Figure 3).[3c] Thus, plasmid DNA

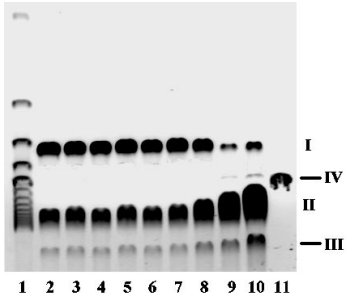


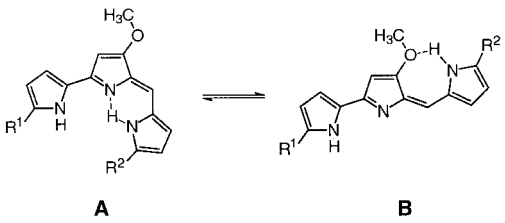
Figure 3. *Result of agarose gel electrophoresis showing the extent of DNA cleavage produced by roseophilin (**1**) and roseophilin analogues **14** – **17** in the presence of $Cu(OAc)_2$ after an incubation time of 60 min at 37 °C. Lane 1: DNA marker (1000 base pairs molecular weight difference); lane 2: DNA alone; lane 3: DNA + **1**; lane 4: DNA + Cu^{II}; lane 5: DNA + **1** + Cu^{II}; lane 6: DNA + **14** + Cu^{II}; lane 7: DNA + **15** + Cu^{II}; lane 8: DNA + **16** + Cu^{II}; lane 9: DNA + **17** + Cu^{II}; lane 10: DNA + **6** + Cu^{II} (positive control); lane 11: linear DNA.*

suffers no appreciable damage if incubated with **1** alone (lane 3), and even a combination of **1** and Cu^{II} is ineffective (lane 5). The same holds true for **14** (lane 6) and **15** (lane 7). Some activity can be noticed for **16** · $Cu(OAc)_2$ (lane 8) and, to a greater extent, **17** · $Cu(OAc)_2$ (lane 9), although it is still lower than that of the prodigiosin complex **6** · $Cu(OAc)_2$ used as a positive control in this experiment (lane 10).

These results allow to draw some important conclusions. It has been inferred from previous studies that the cytotoxic activity of prodigiosin derivatives stems, at least in part, from the pronounced ability of these compounds to cleave double-stranded DNA;[12] in contrast, the structurally related and highly

potent alkaloid roseophilin does not damage this target and, as a consequence, must exert its cytotoxic activity by other mechanisms. Moreover, insights into the structure – activity profiles of such heterocyclic compounds have been gained. Thus, the ability to effect strand cleavage is intimately related to the presence of the intact pyrrolylpyrromethene chromophore. Replacement of either the pyrrole ring A or B of the parent system results in a very significant or even total loss of activity. This is particularly surprising since structural investigations have recently shown that the replacement of the A ring by a benzene unit (**4** versus **11**) does not alter the electron distribution within the heterocyclic domain of such compounds.[10] Furthermore, increasing the electron density by the introduction of appropriate donor substituents (see **17**/**16**/**15**) or by the exchange of a pyrrole for a more electron-rich methoxyfuran unit (**8** versus **15**) decreases rather than increases the potency of these compounds, although such variations facilitate the formation of π-radical cations upon reaction with Cu^{II}. The ability to coordinate metal cations, however, is significantly reduced if one of the pyrrole rings is replaced by another arene.[17] Therefore, we conclude that this parameter is essential for DNA cleavage and, as a likely consequence, for part of the biological activity of prodigiosins.

In contrast to this very conservative pattern concerning the heteroaromatic domain, significant modifications of the alkyl part are well accommodated. It may even be completely absent (→**8**) or can be tied back to form a macrocyclic ring (→**4**) without any appreciable effect on the ability of the compound to effect single- and double-strand cleavage. The latter aspect is particularly noteworthy since prodigiosins usually exist in two different isomeric forms, **A** and **B**, respectively [Eq. (1)].[18] Although in



A B (1)

compound **4**—in contrast to all other derivatives investigated—the *Z* configuration of the azafulvene moiety is locked by the macrocyclic tether,[8b, 10] it shows similar activity as its flexible congeners.

Experimental Section

DNA cleavage assay: Purified scDNA (ca. 300 ng) [ΦX174 RF1 DNA, purchased from MBI Fermentas GmbH, St. Leon-Rot, Germany; the EDTA contained in the commercial sample was removed according to the Qiaex II protocol for desalting and concentrating DNA by using a Qiaex II Gel Extraction Kit] was incubated at 37 °C with the respective pyrrole alkaloid derivative (30 μM final concentration) and $Cu(OAc)_2$ (30 μM) in a solution containing 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (10 mM, pH 7.4), aq. NaCl (75 mM), and MeCN (10%, v/v) (total volume 20 μL) for the time indicated in the figure legends. The mixture was quenched with loading buffer (BioRad laboratories) and the DNA resolved by electrophoresis (Powerpac 300, BioRad) (85 V, 1 h) on a 0.8% agarose gel (containing ethidium bromide) in Tris/boronic acid buffer (BioRad). The bands detected under UV light were analyzed and processed by using the Bio Doc II software (Biometra).

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The Chemistry and Biology of Ratjadone

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KEYWORDS:

antitumor agents · cell cycle · natural products · ratjadones · total synthesis

Ratjadone (**1**), a remarkably cytotoxic secondary metabolite, was isolated in 1994 by Höfle et al. from *Sorangium cellulosum* collected as a soil sample at Cala Ratjada (Mallorca, Spain).[1] It belongs to a family of so-called orphan ligands which include polyketides like leptomycin,[2] callystatin A,[3] and other related compounds.[4] In initial biological evaluations, it was found that ratjadone exhibits high cytotoxicity in cultured mouse cell lines (L929) with an IC_{50} value of 50 pg mL^{-1}. Additionally, it was found that this compound inhibits the growth of the HeLa cell line (KB3.1) at remarkably low concentrations (40 pg mL^{-1}).[5] We initiated the total synthesis of ratjadone in order to provide molecular tools that can be used to investigate the biological effects of individual substructures and to contribute to a better understanding of its mode of action. Our total synthesis of ratjadone was therefore set up to allow the rapid assembly of various ratjadone diastereomers and derivatives from three fragments (Scheme 1).[6a–d] During our manuscript preparation, Williams et al. reported the synthesis of (−)-ratjadone.[6e]

The pivotal steps in the synthesis are a Wittig reaction for the junction of the fragments **B** and **C** followed by a Heck reaction for the attachment to the **A** fragment. For the synthesis of diastereomers, the enantiomeric fragments **A′**, **B′**, and **C′** were synthesized according to our original strategy (Scheme 1). From these different fragments, diastereomeric ratjadone frameworks could be assembled in just two steps, with only three further transformations remaining to obtain ratjadone or any of its diastereomers. By using this strategy, we were able to generate the diastereomeric compounds (**2** – **5**) and analogues (**6** – **9**) shown in Scheme 2.

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