Communications to the Editor

Structure and Function of the Antitumor Antibiotic Carzinophilin A: The First Natural Intercalative Bisalkylator[1]

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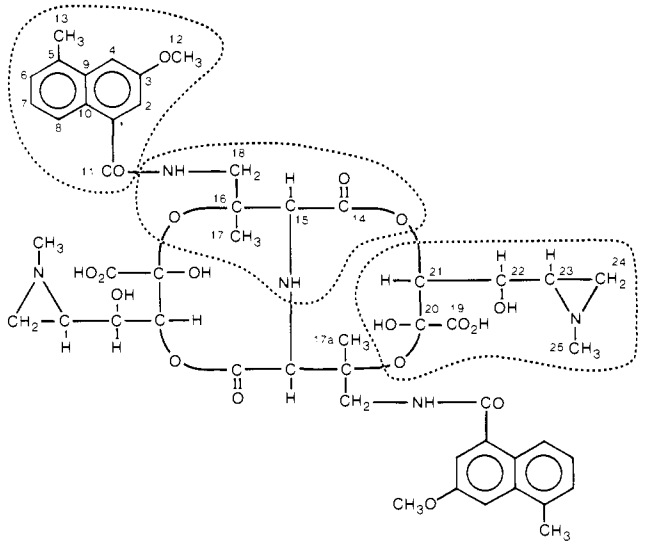
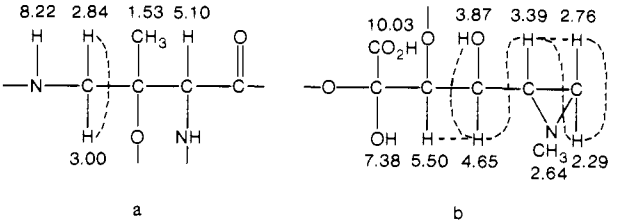
Carzinophilin A (CZ) is an antitumor antibiotic isolated from *Streptomyces sahachiroi*,[2-4] of which only a fragment of the structure has been reported.[5-7] We now describe its complete structure (I) and relative stereochemistry and the relevance of



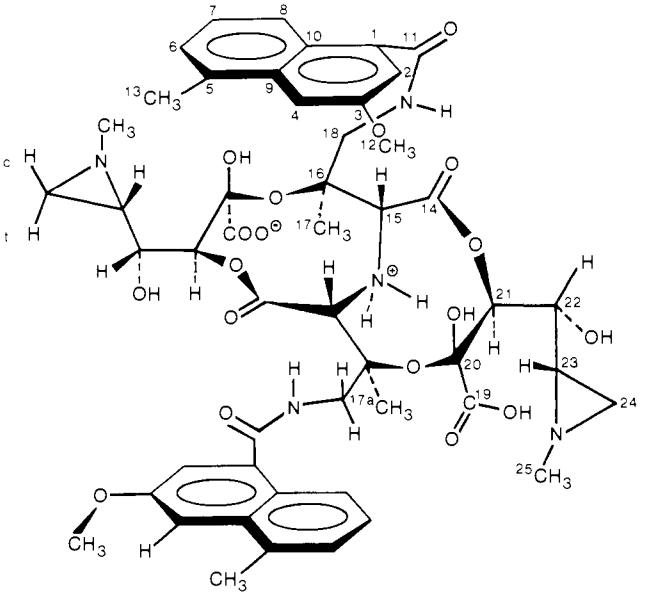
I

these features to the observed DNA interstrand cross-linking[8,9] and antitumor properties of CZ.[3,4] The 400-MHz ^1H NMR spectrum in $CDCl_3$ showed only 29 different protons (molecular formula $C_{50}H_{58}N_5O_{18}$),[6] suggesting the molecule to be dimeric and to possess 2-fold symmetry. The substituted naphthalene chromophore was readily assigned by the characteristic ^1H NMR[6] and ^{13}C NMR chemical shifts. Double resonance of the ^1H NMR spectrum established this unit together with those of a and b with the decoupled protons joined by dotted lines. Hydrolysis of CZ with 5% NH_4OH followed by 20% HCl affords (*S,S*)-*erythro*-4-amino-2,3-dihydroxy-3-methylbutanoic acid, glycine, and 3-methoxy-5-methylnaphthalene-1-carboxylic acid together with an



a b

unidentified basic α-amino acid.[6] It was also shown that the naphthalene moiety was attached to the γ-amino acid by an amide link.[6] Hydrolysis of the nitrogen bridge in I under acidic conditions by an S_N2 process would give a substituted valine and the (*S,S*)-*erythro*-dihydroxy-γ-amino acid observed, requiring an inversion of configuration at C_{15}, which defines the configurations at C_{15} and C_{16} (II). A retro-aldol cleavage between C_{15} and C_{16}



II

during the hydrolysis accounts for the formation of glycine. The basic α-amino acid obtained by Onda et al.[6] would therefore be 2,4-diamino-3-hydroxy-3-methylbutanoic acid.

The stereochemistry of the C_{19}–C_{25} moiety relative to the peptide ring and chromopore was established from the resolution-enhanced spectrum together with NOE difference[10] experiments. The long-range couplings ($J_{21,24cis}$ = 1.5 Hz, $J_{22,24cis}$ = 0.6 Hz) suggest an approximate average coplanarity of the H_{21}–H_{24} fragment. Large NOE differences were found between H_{22} (OH), H_{21} and H_{22}; H_{22}, H_{23}; H_{24cis}, $H_{24trans}$; and H_{23}, H_{24cis}; while a smaller effect for the pair H_{21} and H_{22} was observed. Since in these instances the observation of NOE effects suggests proximity, in contrast the absence of NOE differences for H_{21} with H_{20} (OH) and CH_3(17a) suggests these groups are relatively remote, i.e., trans, which is in accord with the stereochemical assignment in II. The orientation of the C_{20}–OH is confirmed in the dimethyl ester,[11] in which a strong NOE difference between $OCH_{3(12)}$ and H_{20}–OH is observed. These data suggest that the

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C_{19} to C_{24} moiety may be derived biosynthetically from D-glucosamine as is found, for example, in mitomycin C, another aziridine-containing antibiotic.[12]

Spin-echo experiments[13,14] in the ^{13}C NMR of CZ showed positive effects for C_{11}, C_{14}, C_{16}, C_{18}, C_{19}, C_{20}, and C_{24}, confirming that these carbons are directly bonded to zero or two protons, and negative effects for C_{12}, C_{13}, C_{17}, C_{21}, and C_{23}, indicating these carbons are directly bonded to one or three protons, in accord with the assigned structure I.

A zwitterionic form for CZ is anticipated owing to the presence of the carboxyl group at C_{20} and the basic N bridge. Whereas the C_{15}–H appears as a sharp singlet at δ 5.10 in $CDCl_3$, it changes to a triplet (J = 1.5 Hz, δ 5.25) upon addition of CF_3COOH (TFA) when the rate of proton exchange from the bridgehead N is slowed. Upon addition of an equivalent of TFA the aziridine rings may be opened selectively while the peptide backbone of the molecule is unaffected. The direction of the aziridine ring opening as evidenced by the contrasting large chemical shift change for H_{24} (δ 2.5 to 4.50) together with the ^{19}F shifts of the resulting CH_2OCOCF_3 groups (q.v.) corresponds to the S_N2 ring opening expected for a monosubstituted aziridine.[15] During this process the geminal $C_{24}H_2$ coupling of <0.9 Hz, characteristic of an aziridine,[16] becomes the normal value of 12 Hz for an open-chain compound. Treatment with TFA also results in γ-lactone formation between the C_{19} carboxyl and the C_{22}–OH, resulting in the disappearance of the signals due to these two protons, observations of vicinal coupling between H_{21} and H_{22} of 3.7 Hz, and appearance of an IR band at 1750 cm^{-1}.[17] Upon treatment with more than 2 equiv of TFA, the C_{20}–OH and C_{22}–OH groups are acylated, and examination of the ^{19}F NMR spectrum confirms three types of OTFA groups at +0.154, −0.218, and −0.396 (relative to $CF_3CO_2CH_3$), corresponding to terminal (C_{24}) in-chain (C_{22}), and in-ring (C_{20}) OTFA ester groups.[18] That structure I and conformation II indicate similarity to the bis-intercalating quinoxaline antitumor antibiotics (echinomycin,[19] triostin,[19] and luzopeptin[20]) immediately suggests a mode of action for CZ. The intrinsic fluorescence of the naphthalene chromophores[21] (excitation at 346 nm, emission at 427 nm) shows a progressive enhancement up to 200% when aliquots of calf thymus DNA are added to the solution. The presence of the two aziridine moieties in close proximity to these chromophores (II) also accounts for the observed specifically acid-promoted interstrand cross-linking of DNA by CZ.[8,9] These and other aspects of the mechanism of action will be reported subsequently.

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Registry No. Carzinophilin A, 81553-83-5.

Supplementary Material Available: ^1H and ^{13}C NMR data as well as proton coupling constants and spin-echo and nuclear Overhauser differences on carzinophilin A (7 pages). Ordering information is given on any current masthead page.

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(21) Separated by ca. 10.3 Å from a space-filling CPK model, i.e., suitably positioned for bis-intercalation.[19]

Synthesis and Binding Studies of a Photoaffinity Label for Bovine Rhodopsin

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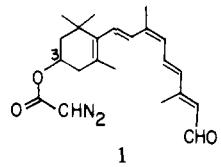
Our earlier studies with various rhodopsins incorporating synthetic model retinals led to the proposal of the external point-charge model[1-3] which accounted for the variation of the absorption maxima of rhodopsins. In the following we report the synthesis of the photoaffinity labeled retinal **1**, properties of the



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bovine rhodopsin formed therefrom, and the fact that the protein binds preferentially to one of the enantiomers at C-3. We believe that such photoaffinity labeling studies[4] will assist in clarifying the positioning of the chromophore within the protein binding site when the apoprotein structure becomes elucidated.[5]

The photoaffinity label should be such that it absorbs light efficiently in a region that overlaps minimally with the pigment absorption, it is introduced at a later stage in the synthesis (for radioactive label studies), and it should be separable from the polyene moiety so that complications arising from double-bond isomerizations etc., are minimized during subsequent sequencing studies. These were the reasons for choosing the diazoacetoxyl group, which had been employed by Westheimer and co-workers in their pioneering studies.[6] The synthesis of the diazoacetate was eventually carried out according to Scheme I. All intermediates and final products were characterized by spectral data.

Dehydro-β-ionone **2**, prepared from β-ionone following known procedures,[7] was converted to 3-hydroxy-β-ionone **3** by hydroboration[8] and then to the 3-hydroxy-9-*cis*-retinal **6** (Scheme I). This was then photoaffinity labeled as follows by slight modification of conventional procedures.

Glyoxylic acid tosylhydrazone[13] (64.5 mg, 0.266 mM) in 300 μL of CH_2Cl_2 and 45 μL of DMF containing (dimethylamino)-pyridine (6.4 mg) was stirred for 15 min at room temperature, and the solution was treated at 0 °C with retinal **6** (80 mg, 0.266

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