Dactylocyclines: Novel Tetracycline Glycosides Active against Tetracycline-Resistant Bacteria

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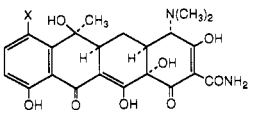
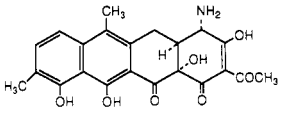
Summary: The dactylocycline antibiotics are a series of tetracycline glycosides with absolute stereochemistry at C-6 (*R*) unprecedented amongst natural tetracyclines which, in addition, display an unusual antibiotic spectrum.

The tetracycline antibiotics once dominated outpatient therapy of bacterial infections but are now less widely used in clinical practice due to the emergence of widespread resistance among pathogens. In an attempt to address this problem, we devised a strategy for detecting novel tetracycline derivatives in nature. Our bioassays included a sensitive screen involving tetracycline (1) reversal of the cetotetrine (2) toxicity toward *Escherichia coli* SC 12623,[1]

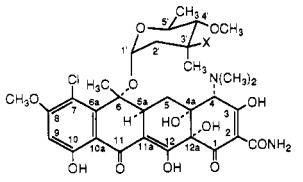


(1) X = H
(9) X = Cl



(2)

a protein synthesis assay based on tetracycline inhibition of penicillin G induced β-lactamase formation by *Bacillus licheniformis* SC 9262, and a battery of tetracycline-sensitive and -resistant microorganisms for determination of antimicrobial spectra. Fermentation broths of *Dactylosporangium* sp. SC 14051 (ATCC 53693), isolated from leaf litter in New Jersey pond water, tested positive in these bioassays. Fractionation of broth extracts using a combination of chromatographies produced several individual dactylocycline components (3–6) which were found to be unusually acid labile and lipophilic as compared to the fermentation-derived tetracyclines of commerce. While it is typical to isolate tetracyclines as hydrochloride salts, the most efficient strategies for purifying dactylocyclines required minimized exposure to acids and yielded antibiotics in their free base forms.[2]



(3) X = NHOH = A
(4) X = NO_2 = B
(5) X = $NHOCOCH_3$ = D
(6) X = OH = E

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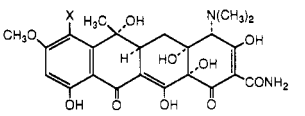
(1) Cetotetrine is a tetracycline antibiotic with an unusual structure whose molecular mode of action involves attack on bacterial membranes, while the classical tetracyclines interfere with ribosomal protein biosynthesis. This is, presumably, the underlying phenomenology for this test. See: Oliva, B. et al. *Antimicrob. Agts. Chemother.* **1992**, *36*, 913–919.

The individual dactylocyclines (Table I) are active in vitro against both tetracycline-sensitive and -resistant Gram-positive microorganisms while being weakly active, at best, against all Gram-negative microorganisms tested. Their aglycon, dactylocyclinone (7) (subsequently iden-



(7) X = Cl
(8) X = H

tified as Sch 34164[3] by comparison of ^1H and ^{13}C NMR data and HPLC coinjection with an authentic sample), on the other hand, is cross-resistant with tetracycline and is more active than the dactylocyclines against Gram-negative microorganisms. Dactylocyclinone is not only found in the fermentation broth but is also produced by mild acid hydrolysis (pH < 4, room temperature) of each dactylocycline. The commonality of aglycon 7 was further demonstrated by MS–MS exact mass measurements on the major daughter ion observed for each dactylocycline. Dactylocycline A was the predominant component in fermentations of the natural strain (as isolated) but better producing subclones of SC 14051 gave a preponderance of dactylocycline B. In extended NMR experiments, air oxidation of dactylocycline A (3) to B (4) was observed, confirming their relationship.

The correct structure of dactylocyclinone (Sch 34164) had been proposed previously[3] and finds further support in our experiments. Unambiguous confirmation of the assignment of the methoxy to C-8 came from ^1H NMR measurements and molecular connectivities shown in 2D-NMR of the relevant atoms in deschlorodactylocyclinone (for 8 in CD_3OD: C-7H, δ 6.78, d, J = 2.4 Hz; C-9H, δ 6.30, d, J = 2.4 Hz), prepared by hydrogenolysis of 7. Likewise, the position of the "extra" (C-4a) hydroxyl was confirmed through inverse detection heteronuclear multiple bond

(2) Yield of individual components varied with each fermentation obtained during the course of this work. The following is typical of the most recent dactylocycline A isolations: Fermentation broth (190 L) was adjusted to pH 5 and extracted with EtOAc. Combined organic extracts were concentrated to an aqueous slurry, applied to Bio-Rad AGMP-50 resin (200–400 mesh, pyridinium form), and after being washed with CH_3CN–H_2O, and antibiotic mixture was eluted with C_5H_5N–CH_3CN–H_2O. The concentrated (not dried) eluate was applied to AGMP-50 resin (Na^+ form) from which antibiotics were eluted with a NaCl–CH_3CN–H_2O) gradient. This ion-exchange chromatography generally separated individual dactylocyclines which were then taken to homogeneity by applying the process described here for dactylocycline A only. Fractions containing dactylocycline A by TLC were filtered through Bio-Rad AGMP-1 resin (100-200 mesh, Cl^- form) and then chromatographed on Mitsubishi CHP20P resin with a CH_3CN–H_2O gradient to yield a yellow amorphous solid upon drying: 48 mg, HRFABMS 698.2314 (calcd 698.2328 for $C_{31}H_{41}N_3O_{13}Cl$), R_f = 0.42 on Merck cyanopropyl TLC plates developed with 3:1 MeOH–0.05 M oxalic acid (adjusted to pH 4.1).

(3) Patel, M.; Gullo, V. P.; Hegde, V. R.; Horan, A. C.; Marquez, J. A.; Vaughan, R.; Puar, M. S.; Miller, G. H. *J. Antibiot.* **1987**, *40*, 1414–1418.

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Table I. Comparative in Vitro Antibiotic Spectra of Dactylocyclines and Dactylocyclinone Determined by Agar Diffusion Assay

antibiotic	HRFABMS MW[a]	amount[b] (μg)	*S. aureus*[c] (tetracycline sensitive)	*S. aureus*[c] (tetracycline resistant)
tetracycline (1)		8	20	9
chlortetracycline (9)		1	17	8
		20	22	13
		63	26	13
dactylocycline A (3)	697.2236	50	19	17
dactylocycline B (4)	711.2001	50	13.5	13
dactylocycline C[d]	727.1990	50	18	17
dactylocycline D (5)	739.2357	50	19	19
dactylocycline E (6)	682.2097	50	20[e]	17[e]
dactylocyclinone (7)	524.1191	22.5	0	

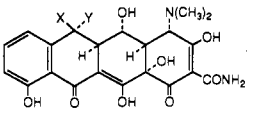
[a] Exact mass of neutral species derived from measurement of MH^+ ion. [b] Applied to 7-mm disks. [c] Zone in mm. [d] The structure of dactylocycline C sugar was not determined. [e] Tested as a mixture with an unidentified analog, MW = 777.

correlation (HMBC) experiments which revealed a network of overlapping correlations (H-4 in each C-4 epimer to C-4a and C-5; H5a to C-5a, C-5, C-6, C-6a and C-6 Me; C-6 Me H to C-6, C-5a and C-6a).[4,5]

The sugar of dactylocycline A was assigned its structure based upon NMR and mass spectrometric measurements of dactylocyclines A and B. Similar sugars are seen in the antibiotics everninomycin[6] and viriplanin.[7] The pyranose H-1 shows no large couplings with either hydrogen at C-2 (broad d, J = 4.6 Hz) and so it must be linked to dactylocyclinone through an α-bond. H-4 is axial relative to H-5 (J = 9.8 Hz, NOE to H-2 and the C-6 Me). The C-3 Me group is axial, based upon its strong NOE to H-5. These assignments are supported by close correspondence of the ^{13}C NMR signals of **3** (C_5D_5N) with those of the sugar from reduced viriplanin ($CDCl_3$). The one exception is the methyl group at C-3 which is, as expected, somewhat less deshielded in the case of dactylocycline A (δ 19.1 vs 23.3). The various assignments of proton and carbon signals are mutually supported by ^1H COSY and HMBC data. The assignment of structure to the sugar of dactylocycline A makes it epimeric at C-3 to the O-desmethylated analog of the sugar from reduced viriplanin. Too little material was available for determination of its absolute configuration which, for the moment, is based on conjecture in formulas **3–6**.

The glycosidic linkage to dactylocyclinone was shown to be at the C-6 hydroxyl from overlapping HMBC data (C-6 methyl hydrogen with C-6 and C-6 with the anomeric proton). This structural assignment is consistent with the extreme acid lability of these glycosides. Dactylocycline A gave a positive Griess test (pyrolysis to HONO), is more basic than dactylocycline B, as expected, and forms a monoacetate (dactylocycline D) on treatment with neat acetic anhydride whereas dactylocycline B does not.

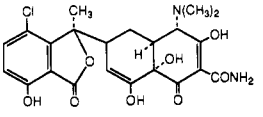
A comparison of the circular dichroism spectra of dactylocycline A, dactylocyclinone and chlortetracycline (**9**) demonstrated identical absolute configurations at C-4, C-4a, C-5a, and C-12a.[8] These intrinsically dissymmetric chromophores dominate the spectrum and the absolute configuration at C-6 cannot be obtained reliably from such measurements. That the absolute configuration at C-6 was reversed as compared to chlortetracycline was first suspected from its unusual chemical shifts in DMSO-d_6 (^1H NMR δ 1.29 for CH_3 whereas chlortetracycline resonates at δ 1.84). These results find analogy in the C-6-deoxy-5-hydroxytetracycline epimers (**10, 11**) where



(10) X = H, Y = CH_3
(11) X = CH_3, Y = H

the α-methyl is observed at 1.7 δ and the β-methyl at δ 1.0.[9] Analogous chemical shift differences are seen in the ^{13}C NMR spectra (DMSO-d_6 δ 25.0 for chlortetracycline and 20.6 for dactylocyclinone).

Chemical support was provided for these spectroscopic inferences. Dactylocyclinone fails to rearrange to an iso derivative with transannular cleavage of the C-ring upon treatment for 20 h at room temperature with 5% aqueous sodium bicarbonate. Under these conditions, chlortetracycline undergoes complete phthalide rearrangement to isochlortetracycline (**12**) as observed through changes



(12)

in UV absorption (from λ_{max} 373 to 306 nm).[10] A molecule with an equatorial hydroxyl group at C-6 would fail to undergo this transformation. Further, dactylocyclinone undergoes dehydration to anhydrodactylocyclinone (**13**) with great difficulty. In the case of chlortetracycline, where a tertiary, benzylic hydroxyl group at C-6 is positioned trans to the C-5a hydrogen, transformation to anhydrochlortetracycline (**14**) occurs in 100 min at room temperature with 6 N HCl and is instantaneous in concentrated sulfuric acid or in methanesulfonic acid (from λ_{max} 373 to 438 nm).[9] In contrast, dactylocyclinone required heating

(4) Epimerization at C-4, a common feature of tetracyclines,[5] is seen through doubling of NMR peaks associated with A-ring atoms and C-5 when dactylocyclinone solutions are allowed to stand.

(5) McCormick, J. R. D.; Fox, S. M.; Smith, L. L.; Bitler, B. A.; Reichenthal, J.; Origoni, V. E.; Muller, W. H.; Winterbottom, R.; Doerschuk, A. P. *J. Am. Chem. Soc.* **1957**, *79*, 2849–2858.

(6) (a) Ganguly, A. K.; Szmulewicz, S.; Sarre, O. Z.; Girijavallabhan, V. M. *J. Chem. Soc., Chem. Commun.* **1976**, 609–611. (b) Ganguly, A. K.; Sarre, O. Z.; McPhail, A. T.; Onan, K. D. *J. Chem. Soc., Chem. Commun.* **1977**, 313–314.

(7) Kind, R.; Hutter, K.; Zeeck, A.; Schmidt-Base, K.; Egert, E. *J. Antibiot.* **1989**, *42*, 7–13.

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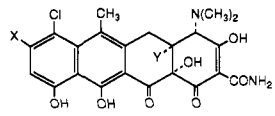
(9) Schach von Wittenau, M.; Blackwood, R. *J. Org. Chem.* **1966**, *31*, 613–615.

(10) Stevens, C. R.; Conover, L. H.; Pasternack, R.; Hochstein, F. A.; Moreland, F. T.; Regna, P. P.; Pilgrim, F. J.; Brunnings, K. H.; Woodward, R. B. *J. Am. Chem. Soc.* **1954**, *76*, 3568–3575.

in methanesulfonic acid for 2 h at 60 °C before dehydration was complete (from λ_{max} 368 to 420 nm). These results find close analogy with similar experiments on C-6 epimers of tetracycline prepared chemically.[11]



(13) X = OCH_3, Y = OH
(14) X = H, Y = H

In sum, the structure and absolute stereochemistry of the dactylocycline antibiotic family is proposed. These interesting molecules are the first known tetracycline glycosides and the first naturally occurring C-6 *epi* tetracyclines. Apart from their intrinsic biogenetic novelty, the dactylocyclines display potentially important activity against tetracycline-resistant microorganisms. The significance of the glycosyl group to the antimicrobial properties of the dactylocyclines is now under study.

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Supplementary Material Available: UV and CD data for 7 and 9 and ^1H and ^{13}C NMR data for 3, 7, and 8 (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(11) Martell, M. J., Jr.; Ross, A. S.; Boothe, J. H. *J. Am. Chem. Soc.* **1967**, *89*, 6780–6781.