DACTYLOCYCLINES, NOVEL TETRACYCLINE DERIVATIVES PRODUCED BY A *Dactylosporangium* sp.

III. ABSOLUTE STEREOCHEMISTRY OF THE DACTYLOCYCLINES

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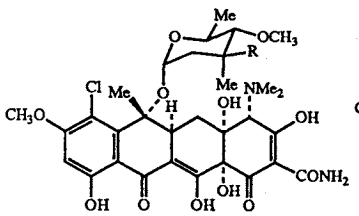
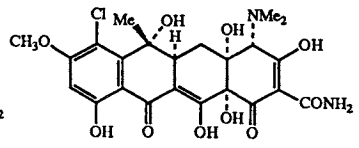
The dactylocycline antibiotics were found through circular dichroism measurements, NMR spectroscopy and chemical transformations to possess the usual tetracycline family absolute configuration at carbons 4, 4a, 5a and 12a. The absolute stereochemistry about the C-6 carbon, however, was the reverse of that found with previously investigated tetracyclines.

The tetracycline antibiotics once dominated outpatient antibiotic care but have declined significantly in popularity of late due to widespread bacterial resistance. It is generally accepted that this resistance is due to evolutionary pressures. This leads to the interesting hypothesis that counter-evolutionary pressures on the part of producing organisms could lead to biosynthesis of naturally occurring tetracyclines which would, in turn, escape these resistance mechanisms. If so, screening programs should be able to identify such molecules and they might be suitable choices for chemotherapy in their own right or, failing this, their molecular identity should provide clues for chemists to follow in overcoming the resistance phenomenon through partial or total chemical synthesis. The properties of the products of such a screening program, the dactylocyclines, glycosides of Sch 34164[1] (now named dactylocyclinone), are described in the preceding papers.[2,3] In this paper we complete the chemical characterization of these novel substances by determining their absolute configuration.

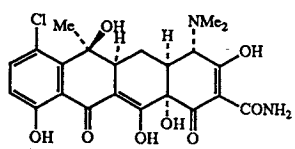
The near correspondence of the ultraviolet/visible spectra of the dactylocyclines (**1**) and dactylocyclinone (**2**) with chlortetracycline (**3**) indicate a close similarity of their chromophores (Fig. 1). Circular dichroism (CD) measurements of a variety of tetracyclines have confirmed that two chromophoric



1 Dactylocycline A: R=NHOH
Dactylocycline B: R=NO_2



2 Dactylocyclinone



3 Chlortetracycline

Fig. 1. Ultraviolet/visible absorption spectra of chlortetracycline (solid line) and dactylocyclinone (dashed line).

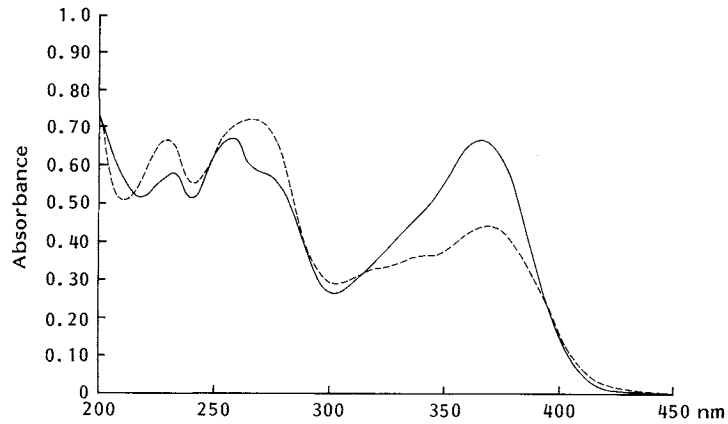
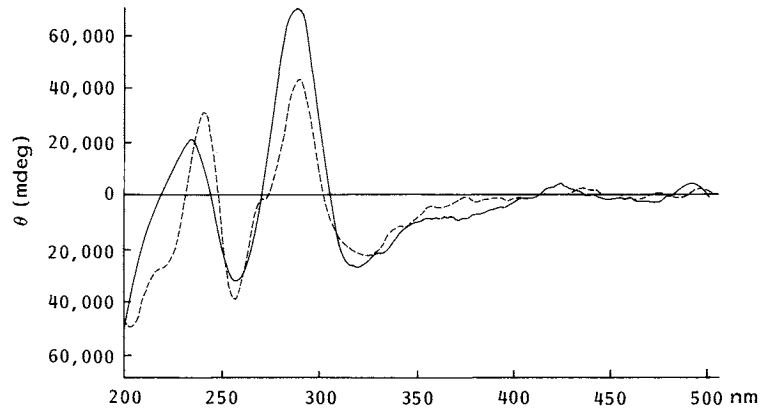
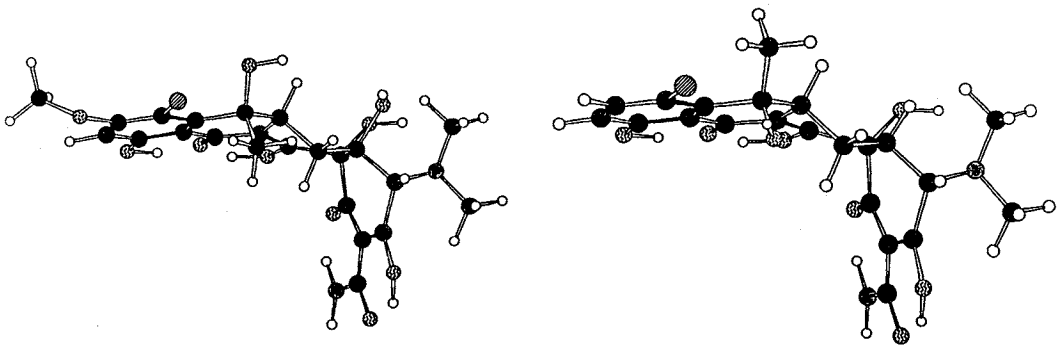


Fig. 2. Circular dichroism spectra of chlortetracycline (solid line) and dactylocyclinone (dashed line) in 0.03 N hydrochloric acid solutions.



regions are present, that each are intrinsically dissymmetric, and that the solution conformation of the various naturally occurring classic tetracyclines is essentially the same.[4~7] The CD spectra of dactylocyclinone and chlortetracycline are reproduced in Fig. 2. The A ring chromophore includes the triacyl system belonging to carbons 1~3 and their pendant functional groups and is twisted due to the 1,3-diaxial interaction of the C-12a hydroxyl and the C-4 dimethylamino moiety (Fig. 3). This chromophoric region gives rise to an intense Cotton effect in the 270 nm region.[7] The BCD chromophore includes the aromatic D ring and the conjugated β-dicarbonyl system extending from carbons 11~12. This chromophore is twisted due to the tetrahedral nature of carbon 5a and gives rise to a series of bands located roughly at 360, 320 (shoulders), 260 and 230 nm.[7] These two chromophores are kept from interacting through the carbon framework by the quaternary carbon bearing the hydroxyl group at C-12a. The *cis*-AB ring juncture, however, fixes these two chromophores so that bands of roughly equivalent energy content can mix through space in the manner known as exciton coupling.[8,9] This is detected in the naturally occurring ring AB *cis* series by two oppositely signed extrema centered roughly about 275 nm such that a positive band at about 290 nm is followed by a negative band at about 260 nm.

Fig. 3. Conformational drawings of dactylocyclinone and chlortetracycline viewed from the side bearing the chlorine and dimethylamino groups.



Dactylocyclinone Chlortetracycline

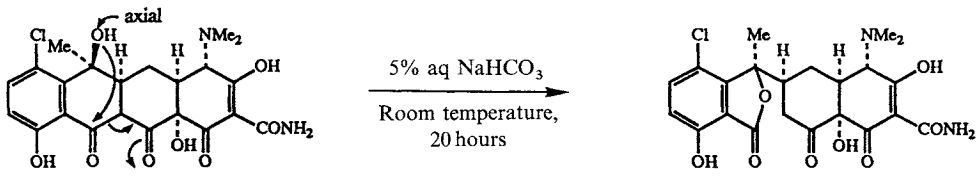
This sign sequence is consistent with the absolute configuration assigned to the tetracyclines by degradation studies.[10] When the C-4 dimethylamino group epimerizes, the degree of twisting of the A ring chromophore is dramatically decreased and the intensity of this exciton band is greatly reduced.[7] When the orientation of the hydrogen at C-5a is inverted, the sign of the BCD ring peaks is inverted as the molecule reverses its twist.[5] The presence of exciton coupling of the same sign in the major component indicates that the AB ring juncture is not only *cis* in the dactylocyclines but is orientated in the same absolute sense as it is in chlortetracycline. Thus the similarity of the CD spectra of dactylocycline A (in methanol to minimize glycolysis) to that of dactylocyclinone and, in turn, to chlortetracycline (Fig. 2) requires that the sugar not be attached to the chromophore, that its presence not affect dramatically the conformation and that the absolute configuration of carbons 4, 4a, 5a and 12a be the same as is found in chlortetracycline.

Given what is known about the influence of stereochemistry on antibiotic activity in the tetracycline family[11] this is hardly surprising but it is gratifying to have objective evidence for this.

The relative and absolute stereochemistry at the C-6 position is, however, not so easily established. The orientation of C-6 substituents has little effect on the conformation and, particularly, the degree of twisting of the intrinsically dissymmetric ring BCD chromophore. The most prominent manifestation of this is the minor influence on circular dichroism spectra of epimerization at C-6.[4,5] Thus evidence of a different nature is required to establish the absolute stereochemistry at the remaining center in the basic ring system.

The nuclear magnetic resonance spectra of dactylocyclinone indicated that something unusual was present at C-6. The C-6 methyl group in tetracycline resonates (in methanol) at δ 1.60 whereas the corresponding group in dactylocyclinone resonates at δ 1.25. In the C-6-deoxy-5-hydroxytetracycline series, the methyl group with the natural alpha-configuration resonates at δ 1.7 while its epimer (β-methyl) resonates at δ 1.0.[12] This shift on epimerization is associated with the relationship of the methyl group to the ring current of the adjoining aromatic ring with the equatorial methyl group being more deshielded. This suggested strongly that the absolute stereochemistry of the dactylocyclines at C-6 be reversed compared with that of all precedent fermentation-derived tetracyclines. A supportive comparable shift in resonances was seen in the carbon spectra (δ 22.9 for tetracycline and δ 20.6 for

Fig. 4. The rearrangement of chlortetracycline to isochlortetracycline in 5% aqueous sodium bicarbonate solution.



Chlortetracycline, λ_{373}

4 Isochlortetracycline, λ_{306}

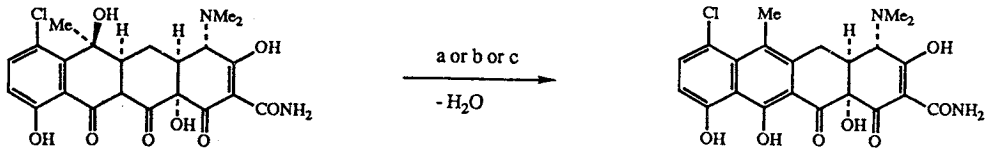
dactylocyclinone). Unfortunately, the epimeric deoxytetracyclines were prepared before carbon spectrum measurements were available so reference material is not available for comparison.

Given the unprecedented nature of this inferred stereochemistry, it was desired to have chemical proof to supplement the spectroscopic findings. Chlortetracycline is well known to undergo transannular ring cleavage in base to form isochlortetracycline (**4**).[13] This rearrangement results in a dramatic change in the BCD ring chromophore and is readily detected in the ultraviolet/visible spectrum (373 nm→306 nm; Fig. 4). In our hands chlortetracycline underwent this conversion smoothly and completely in 20 hours in 5% aqueous sodium bicarbonate solution at room temperature. The chromophore of dactylocyclinone was completely unaffected by this treatment (368 nm→368 nm). If the C-6 hydroxyl group of dactylocyclinone were, indeed, equatorial, then this would be the expected result.

In chlortetracycline, the C-6 hydroxy group is benzylic, tertiary, axial and antiperiplanar *trans* to the C-5a hydrogen. Thus it rapidly undergoes dehydration and aromatization to the corresponding anhydrochlortetracycline (**5**) under comparatively mild acidic conditions.[13] This change dramatically affects the ring BCD chromophore and is easily detected visibly and in the UV/vis spectrum. In our hands, chlortetracycline completely underwent this change in 100 minutes in 6 N HCl (373 nm→438 nm) and instantaneously in concentrated sulfuric acid and in methanesulfonic acid (Fig. 5). The new chromophore was stable for at least 24 hours in concentrated sulfuric acid. As would be expected from its altered stereochemistry, dactylocyclinone formed an anhydro analog only with great reluctance. In 6 N HCl, there was no change in the chromophore of dactylocyclinone in 12.5 hours at room temperature followed by heating at 90°C for four hours (368 nm→368 nm). There was a slow change to a new chromophore (but not an anhydrotetracycline) in concentrated HCl following 96 hours at reflux (368 nm→363 nm). In concentrated sulfuric acid another new chromophore was formed which was not an anhydrotetracycline and this chromophore was stable for 22 hours at room temperature (368 nm→382 nm). Successful conversion of dactylocyclinone to an anhydrotetracycline (anhydrodactylocyclinone, **6**) was finally achieved by heating in methanesulfonic acid for two hours at 60°C (368 nm→420 nm). The extreme degree of this resistance to dehydration was not expected but is consistent with reverse stereochemistry at C-6 as concerted dehydration processes are no longer possible. The very limited amounts of material available precluded structural characterization of the substances with visible maxima at 363 and 382 nm.

The C-6 stereochemistry that these experiments require for the dactylocyclines is not a consequence of the loss of the sugar. It is not credible to believe that the sugar would have been lost from such a hindered position by a Walden inversion process. It is also not credible that the sugar would have been lost through an E-1 process involving an intermediate carbonium ion at C-6 because this should then

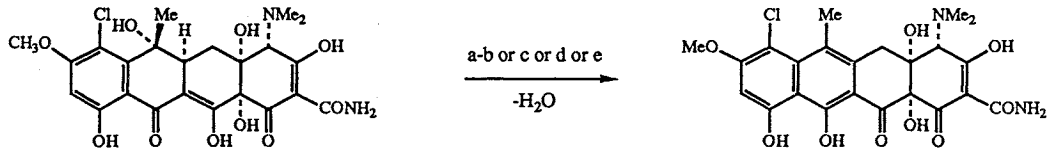
Fig. 5. The acid-catalyzed transformation of chlortetracycline to anhydrochlortetracycline and of dactylocyclinone to anhydrodactylocyclinone and other, as yet unidentified, transformation products.



Chlortetracycline, λ_{373}

5 Anhydrochlortetracycline, λ_{438} $\Delta\lambda = 55$ nm

a = 6N HCl 100 minutes for complete conversion at room temperature.
b = conc H_2SO_4 Reaction complete instantaneously. Stable for at least 24 hours.
c = $MeSO_3H$ Reaction complete instantaneously at room temperature.



Dactylocyclinone, λ_{368}

6 Anhydrodactylocyclinone, λ_{420}

a = 6N HCl No reaction in 12.5 hours at room temperature.
b = 6N HCl No reaction in 4 hours at 90°C.
c = conc HCl Conversion to a new chromophore (but not an anhydro derivative, $\lambda_m = 363$ nm) in 96 hours at reflux.
d = conc H_2SO_4 New chromophore, $\lambda_m = 382$ nm. Stable for 22 hours at room temperature.
e = $MeSO_3H$ Anhydro derivative formed at 60°C in 2 hours, $\lambda_m = 420$ nm.

have resulted rapidly in aromatization to anhydrodactylocyclinone (**6**) rather than interception by water to produce a C-6 epimer with no sign of the normal anhydro product. The conclusion is inescapable that the dactylocyclines are epimeric at C-6 to the precedent naturally occurring tetracyclines as the NMR measurements require.

The β-orientation of the linkage of the sugar to the C-6 hydroxyl group was demonstrated in the previous paper by ^1H NMR measurements.[3] The data available to date does not allow an experimentally based assignment of stereochemistry to the sugars of these novel antibiotics. The quantities available were too small for isolation and purification of the individual sugars so their absolute stereochemistry rests at present on the presumption of an analogy to the everninomycins and viriplanin.

The "extra" features of the dactylocyclines and the corresponding aglycones (such as Sch 34164), although barely precedented, are consistent with what is known about the biosynthesis of the tetracyclines.[14] The C-8 methoxyl moiety presumably stems from retention and methylation of one of the polyketide oxygens which is normally lost by reduction and dehydration in the biosynthesis of the tetracyclines of commerce. The oxygen atom at C-6 is the consequence of a late event taking place at a methylene derived carbon, although in *Dactylosporangium* sp. ATCC 53693 it must take place from the opposite face compared with the tetracycline-producing *Streptomyces* species. The C-4a hydroxyl group must be posited as resulting from a late step also because the oxygen normally present at this carbon in the polyketide is consumed in forming the AB ring juncture. The lability of the glycoside linkage and the lateness of introduction of the C-6 hydroxyl itself suggest strongly that glycosylation must also be a late step. None of these steps, however unusual in the tetracycline family, is unreasonable biosynthetically.

Experimental

Proton and carbon nuclear magnetic resonance spectra were recorded with a Bruker AM-500 (500 MH) spectrometer using trimethylsilane as the internal reference. Ultraviolet spectra were recorded on a Hewlett-Packard Diode Array 8450A instrument. Circular dichroism spectra were recorded with an AVIV Model 60DS spectrometer.

Base Rearrangement and Acid Dehydration Experiments

The acid dehydration and sodium bicarbonate reactions were performed with 1 mg of the corresponding tetracycline analog and 0.5 ml of the appropriate reagent. An aliquot of the reaction mixture was withdrawn periodically, diluted with UV-grade MeOH, and the ultraviolet-visible spectrum recorded.

Chlortetracycline (MeOH - HCl): 232 (ε=39,225), 258 (39,925), 266 (39,975) and 373 nm (29,800); (0.03 N HCl): 226 (ε=18,220), 262 (17,575) and 368 nm (10,177).

Tetracycline (MeOH): 218 (ε=13,950), 270 (15,950) and 369 nm (12,790).

Dactylocycline A (MeOH): 240 (ε=17,950), 261 (18,290) and 371 nm (18,795).

Dactylocyclinone (MeOH - HCl): 233, 259 and 368 nm; (MeOH): 234 (ε=20,075), 258 (18,890) and 371 nm (19,760); (0.03 N HCl): 230 (ε=21,340), 255 (21,800), 274 (18,110) and 363 nm (19,951).

Circular Dichroism Spectra of Various Relevant Tetracyclines

The circular dichroism spectra were measured under the conditions described above and are reported as $[\theta] \times 10^{-4}$.

Tetracycline (MeOH): $[\theta]$ 204 (−2.38), 233 (+0.65), 244 (+0.30), 287 (+4.63), 323 (−1.24), 388 (−0.88).

Chlortetracycline hydrochloride (0.03 N HCl): $[\theta]$ 233 (+2.01), 258 (−3.12), 287 (+7.0), 319 (−2.62).

Dactylocycline A (MeOH): $[\theta]$ 204 (−8.99), 243 (+2.28), 258 (−2.75), 278 (+5.87), 359 (−1.57).

Dactylocyclinone (MeOH): $[\theta]$ 241 (+1.8), 257 (−1.62), 290 (+3.88), 326 (−1.59), 393 (+0.98).

Dactylocyclinone (0.03 N HCl): $[\theta]$ 203 (−4.91), 240 (+3.01), 257 (−3.78), 289 (+4.25), 324 (−2.20).

Acknowledgments

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