X-RAY CRYSTALLOGRAPHY OF PROTYLONOLIDE AND ABSOLUTE CONFIGURATION OF TYLOSIN

Sir:

Protylonolide, a 16-membered lactone, is a metabolite of a mycaminose idiotroph obtained from *Streptomyces fradiae* KA-427[1,2], a producer of the 16-membered macrolide antibiotic, tylosin. The metabolite is converted to tylosin when added to the cultures of the parent and tylosin-non-producing blocked mutants[1]. In the present paper we report the elucidation of the structure of protylonolide by X-ray crystallography, and hence propose the absolute configuration of tylosin.

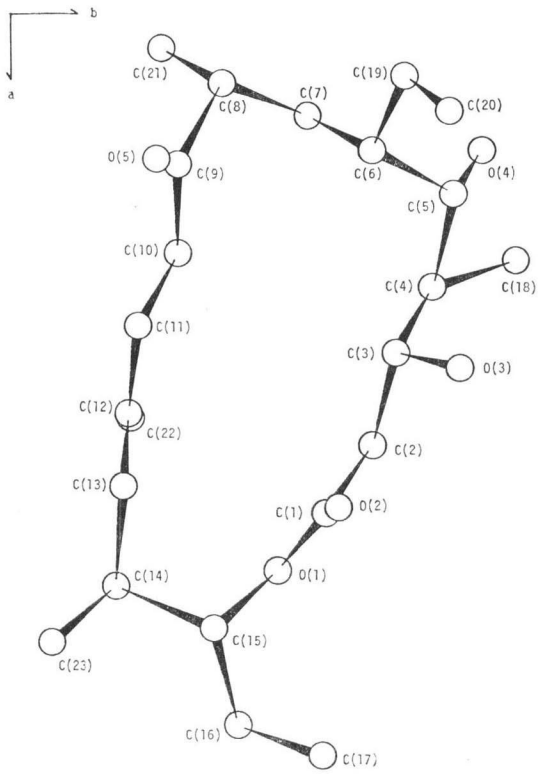
Crystal data: $C_{23}H_{38}O_5$, m.p. 53~56°C, orthorhombic, space group $P2_12_12_1$, $a=13.594$ (3), $b=15.763$ (5), $c=11.104$ (2) Å, $V=2379.4$ Å^3, $Z=4$, $D_{obsd.}=1.11$ gcm^{-3}, $D_{cald.}=1.101$ gcm^{-3}. Intensity data ($2\theta<125°$) were collected from a crystal $0.3\times0.3\times0.5$ mm^3 on an automatic, four-circle diffractometer using graphite monochromated Cu $K\alpha$ radiation. For the structure determination, 1,240 independent structure factor amplitudes greater than their estimated standard deviations were selected, and the structure was solved by the Monte Carlo direct method[3] using the 40 strongest reflections as the starting set, Least-squares refinement was repeated and the final R value was 13.1%. The atomic coordinates are shown in Table 1. The structure of protylonolide thus obtained is shown in Fig. 1.

We have reported that the configurations at C-3, C-4, C-5, C-6 and C-8 in the aglycone of tylosin are *R, S, S, R* and *R*, respectively, in comparison of CD and ^{1}H-nmr data of derivatives of tylosin with those of leucomycin, a 16-membered

Table 1. Atomic coordinates of protylonolide. Standard deviations are referred to the last digits.

Atom	X	Y	Z
O(1)	0.4518(4)	0.5613(4)	0.1723(6)
O(2)	0.3839(4)	0.6203(4)	0.3325(6)
O(3)	0.2341(4)	0.7418(3)	0.2943(6)
O(4)	−0.0197(4)	0.7675(4)	0.0796(6)
O(5)	0.0053(4)	0.4455(4)	0.3567(6)
C(1)	0.3910(6)	0.6101(6)	0.2229(8)
C(2)	0.3184(6)	0.6584(5)	0.1449(9)
C(3)	0.2172(6)	0.6808(5)	0.2029(9)
C(4)	0.1448(6)	0.7167(5)	0.1084(9)
C(5)	0.0443(6)	0.7363(5)	0.1696(9)
C(6)	−0.0030(7)	0.6580(5)	0.2383(10)
C(7)	−0.0400(6)	0.5950(5)	0.1422(9)
C(8)	−0.0760(6)	0.5094(5)	0.1850(9)
C(9)	0.0101(8)	0.4662(5)	0.2461(10)
C(10)	0.1044(6)	0.4492(6)	0.1812(9)
C(11)	0.1875(6)	0.4261(5)	0.2354(9)
C(12)	0.2824(6)	0.4177(5)	0.1738(9)
C(13)	0.3614(7)	0.4116(5)	0.2495(9)
C(14)	0.4702(7)	0.4065(6)	0.2227(9)
C(15)	0.5150(7)	0.4994(6)	0.2375(9)
C(16)	0.6216(7)	0.5216(6)	0.2026(10)
C(17)	0.6549(6)	0.6045(6)	0.2439(11)
C(18)	0.1890(8)	0.7982(6)	0.0531(9)
C(19)	−0.0835(6)	0.6904(6)	0.3255(11)
C(20)	−0.0470(7)	0.7331(7)	0.4409(9)
C(21)	−0.1142(7)	0.4512(6)	0.0887(9)
C(22)	0.2880(7)	0.4189(7)	0.0370(8)
C(23)	0.5306(7)	0.3382(5)	0.2767(11)

Fig. 1. *c*-Axis projection of protylonolide.



moieties, mycaminose, mycarose and mycinose, has been determined[5,6], the determination of the absolute configuration of tylosin has been completed (Fig. 2). The stereochemistry of tylosin agrees with CELMER's empirical model[6] for 16-membered macrolide aglycone, except for the configuration at C-14. More recently, a similar conclusion was drawn[7] in the case of acumycin which is one of the tylosin subgroup of 16-membered macrolides[8]. CELMER's model was empirically proposed, based on the stereochemistry of oleandomycin and erythromycin and has been well accepted in the case of 12- and 14-membered macrolide series. However, one exception, that of the stereochemistry at C-14 of the present compound refuses application of the model to 16-membered macrolides.

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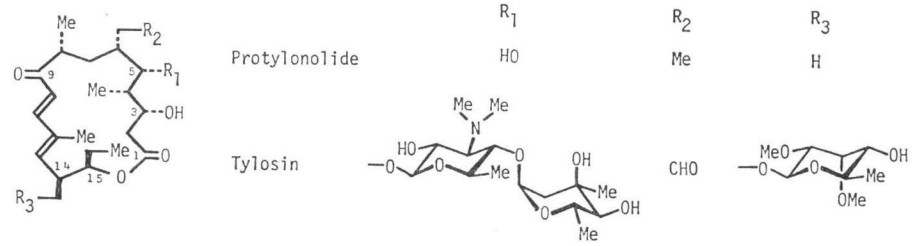
Fig. 2. Absolute configuration of protylonolide and tylosin.



macrolide for which the absolute configuration was already established[4]. The X-ray analytical data for protylonolide does not contradict with the previous conclusion on the stereochemistry of the aglycone of tylosin. From the evidences that protylonolide was bioconverted to the aglycone of tylosin and the absolute configurations of five carbons described above has been determined, we can now show that both configurations at C-14 and C-15 are *S* and *R*, respectively. We can also confirm that the diene system at C-10, C-11, C-12 and C-13 has the *trans-trans* configuration. Since the stereochemistry of three sugar

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