CONGLOBATIN, A NOVEL MACROLIDE DILACTONE FROM *STREPTOMYCES CONGLOBATUS* ATCC 31005

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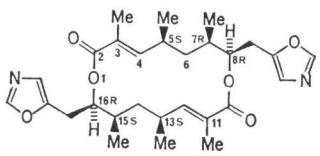
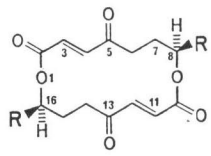
Fermentation of deposited cultures of *Streptomyces conglobatus*, known to produce the polyether antibiotic, ionomycin has resulted in the isolation and characterization of a second metabolite, conglobatin ($C_{28}H_{38}N_2O_6$). X-Ray analysis revealed a dimeric macrolide dilactone structure for conglobatin, similar to the structures of the mold metabolites vermiculin and pyrenophorin, from which the absolute configuration of conglobatin has been inferred. The dimer consists of two molecules of 7-hydroxy-8-oxazoyl-2,4,6-trimethyl-2-octenoic acid joined by two ester linkages.

As part of our continuing interest in polyether antibiotics[1], a culture of *Streptomyces conglobatus* sp. nov. TREJO (ATCC 31005) known[2] to produce ionomycin was acquired and fermented as described earlier by W.-C. LIU, *et al.*[3] The resulting ionomycin was isolated and characterized as a calcium ionophore[4] with the unique ability of forming a 1:1 complex with that divalent cation.

This report describes the isolation from the same culture of *S. conglobatus* of a novel macrolide dilactone, conglobatin (**I**) which in our hands is produced at a twenty times higher yield than ionomycin.



I



IIa: R = CH_2COCH_3

IIb: R = CH_3

Isolation of Conglobatin from Fermented Cultures of *Streptomyces conglobatus* ATCC 31005

The isolation, separation from ionomycin, and purification of conglobatin was carried out as summarized in Scheme 1. The isolated yield of crystalline conglobatin was equivalent to 350 mg/liter.

Scheme 1. Isolation of conglobatin

Whole broth (105 gallons)
↓ extracted with ethyl acetate and the extract concentrated.
Syrup (9 kg)
↓ Partitioned between acetonitrile and *n*-hexane. The acetonitrile layer was concentrated.
Viscous oil (750 g)
↓ Extracted with ether, in which ionomycin (6 g) is insoluble. Ether extract concentrated.
Crude conglobatin (383 g)
↓ Dissolved in ether - *n*-hexane (3:1) and the solution evaporated.
Crystalline conglobatin (136 g)

Physical and Chemical Properties

Crystalline conglobatin melts at 125°C and has an optical rotation in chloroform, $[\alpha]_D$ −44° (*c* 1). Mass spectrometry yielded a molecular

ion at *m/e* 498 and this combined with microanalysis gave a molecular formula of $C_{28}H_{38}N_2O_6$ (498.62).

Calculated for $C_{28}H_{38}N_2O_6$: C, 67.45; H, 7.68; N, 5.62
Found: C, 67.54; H, 7.84; N, 5.64

The infrared absorption spectrum of conglobatin is shown in Fig. 1 with peaks at 1705, 1275 (α, β-unsaturated ester), 1650 (C=C), 1610, 1505 (aromatic) cm^{-1} in KBr. The ultraviolet spectrum in ethanol displays a maximum at 214 nm (ε 43,800) which confirms the presence of an α,β-unsaturated ester or lactone.

The proton NMR spectrum (Fig. 2) consists of peaks at δ 0.98 (6H, d, $\underline{CH_3}CH$, J=6.5 Hz), 1.10 (6H, d, $\underline{CH_3}CH$, J=6.5 Hz), 1.28, 1.42, 1.71 (6H, m, $CH_3\underline{CH}$), 1.74 (6H, s, $\underline{CH_3}C{=}CH$), 2.55 (2H, m, $\underline{CH}{-}CH{=}$), 2.86 (4H, d, CH_2, J=6 Hz), 5.17 (2H, m, CH=), 6.32 (2H, d, CH=, J=10 Hz), 6.78 (2H, s, CH=) and 7.75 (2H, s, CH=). The ^{13}C-NMR gave a fourteen peak spectrum consistent with a symmetrical dimeric C_{28} structure.

Fig. 1. IR Spectrum of conglobatin (KBr).

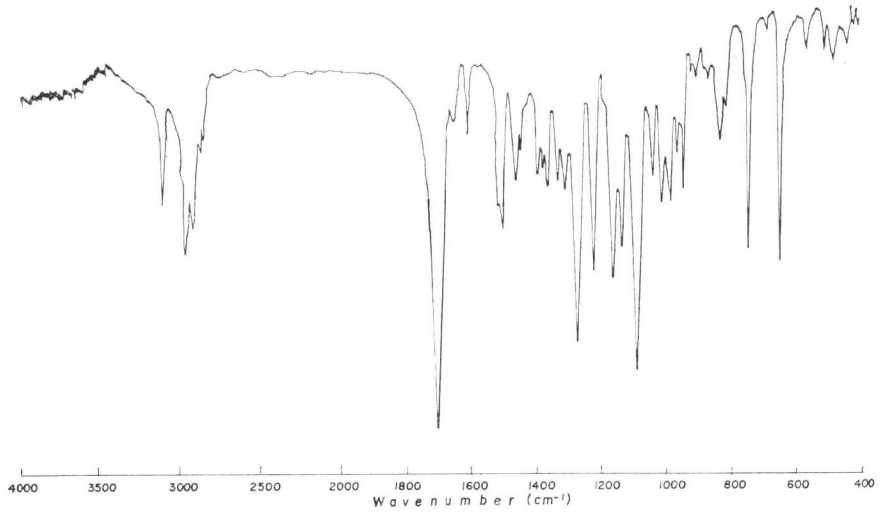
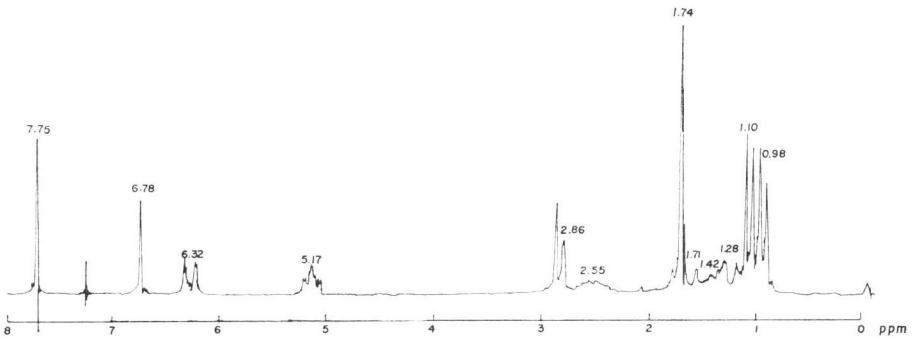


Fig. 2. 100 MHz in $CDCl_3$ NMR spectrum of conglobatin.



Structure of Conglobatin

The structure of conglobatin was determined by a single crystal X-ray analysis and a stereoscopic drawing of the molecule is shown in Fig. 3. The crystal data are summarized in Table 1. Intensities were determined using a Hilger-Watts diffractometer (Ni filtered Cu Kα radiation, $\theta-2\theta$ scans, pulse height discrimination). The size of the crystal used for data collection was approximately $0.05\times0.15\times0.45$ mm; the data were not corrected for absorption. Of the 3318 accessible reflections for $\theta<76°$, 2359 were considered to be observed [$I>2.56$ (I)]. From these data, the structure was solved by a multiple solution procedure[5] and was refined by full-matrix least squares. In the final refinement, anisotropic thermal parameters were used for the heavier atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indexes were $R=0.057$ and $wr=0.070$ for the 2359 observed reflections. The final difference map had no peaks greater than ±0.2 eA^{-3}.

Fig. 3. Stereoscopic view of conglobatin in the crystalline state.

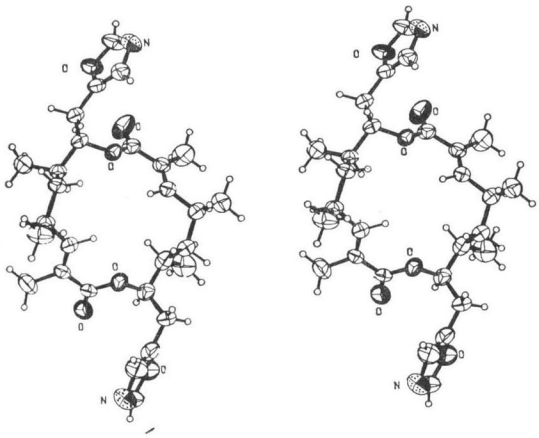


Table 1. Conglobatin: Crystal data

Formula	$C_{28}H_{38}N_2O_6$
Formula weight	498.62
Space group	$P2_1$
a	10.356 (3) A
b	6.202 (2) A
c	23.334 (5) A
β	100.94 (2)°
Z	2
Density (calc.)	1.125 g cm^{-3}
μ(Cu Kα)	6.6 cm^{-1}

The structure elucidated by X-ray analysis could only indicate the relative configuration of the six asymmetric centers present in the conglobatin molecule. The absolute configuration has been assigned in structure **I** by analogy with (−)-vermiculin (**IIa**) produced by *Penicillium vermiculatum*[6] and (−)-pyrenophorin (**IIb**) produced by *Stemphylium radicinum*[7]. As in the case of **I**, X-ray analysis of **IIa**[8] yielded only the relative configuration. The absolute configuration has been determined, however, for both **IIa** and **IIb** by stereospecific syntheses[9] via an intermediate of known configuration which yielded the unnatural (+)-enantiomers. The absolute configuration of (−)-vermiculin has recently been confirmed, as represented by **IIa**, by total synthesis[10].

The systematic name of **I** is [5S, 7R, 8R, 13S, 15R, 16R]-3,5,7,11,13,15-hexamethyl-8,16-bis (oxazol-5-ylmethyl)-1,9-dioxacyclohexadeca-3,11-diene-2,10-dione and the compound presumably arises biosynthetically by the dimerization of [2,4S,6R,7R]-7-hydroxy-8-oxazoyl-2,4,6-trimethyl-2-octenoic acid.

Conglobatin was not toxic at doses up to 1 g/kg when administered to mice either orally or interperitoneally, but did not exhibit any of the antifungal, antibacterial, antiprotozoal[6] or antitumor activity[11] claimed for (−)-vermiculin.

Acknowledgements

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