Tetrahedron Letters No.2, pp. 91-95, 1969. Pergamon Press. Printed in Great Britain.

TOLYPOMYCIN. I. STRUCTURE OF TOLYPOMYCINONE

T. Kishi, M. Asai, M. Muroi, S. Harada,
E. Mizuta, S. Terao, T. Miki and K. Mizuno
Research and Development Division, Takeda Chemical Industries, Ltd.,
Higashiyodogawa-ku, Osaka, Japan

(Received in Japan 5 November 1968; received in UK for publication 4 December 1968)

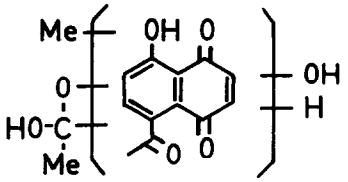
Tolypomycin Y (I) is a new antibiotic obtained as a metabolite of Streptomyces tolypophorus (1), which exhibits a strong inhibition against Gram-positive bacteria. Mild acid hydrolysis (1 Mol. HCl in 70% MeOH) of I, $C_{43}H_{54}N_2O_{14}$, afforded a quinone, tolypomycinone (II), $C_{37}H_{43}NO_{13}$, $[\alpha]_D$ +350° in MeOH, UV λ_{max}^{EtOH} mμ (ε): 228 (32,100), 278 (20,900), 307 (15,500), 396 (5,100), IR (KBr, cm^{-1}): 1740 (-OCO), 1715 1705 (CO), 1680 (CO), 1655, 1640 (CO, CONH), NMR*1 ($CDCl_3$, δ value (ppm)): 0.19, 0.65, 1.12 (each 3H, d, CH-Me), 1.73 (3H, s, -Ċ-Me), 1.99 (3H, s, OAc), 2.12 (3H, d, CH=C-Me), 2.33 (3H, s, φ-Me), 3.09 (3H, s, OMe), 12.6 (1H, s, chelated OH), and tolyposamine (III) (2). On catalytic reduction, II gave dihydrotolypomycinone (IV), UV λ_{max}^{EtOH} mμ (ε): 229 (39,800), 303 (20,800), 444 (9,300) and tetrahydrotolypomycinone (V), UV λ_{max}^{EtOH} mμ (ε): 233 (26,200), 302 (19,600), 441 (9,200). Mild oxidation of V with ferric chloride yielded another dihydrotolypomycinone (VI), UV λ_{max}^{EtOH} mμ (ε): 228 (21,900), 277 (18,600), 302 (sh.), 332 (8,200), 407 (3,900). The characteristic relationships between the two quinones, II and VI, and two hydroquinones, IV and V, were observed in their UV-spectra and oxidation-reduction reactions.

Further hydrolysis of II with acid (60% acetone-0.5N-HCl) under somewhat rigorous conditions afforded a red quinone, tolyponone (VII), $C_{14}H_{10}O_7$,

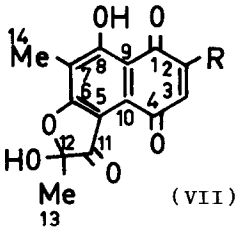
*1 d = doublet, s = singlet, t = triplet, q = quartet, m = multiplet

mp 198° (decomp.), IR (KBr, cm^{-1}): 1685 (C=O), 1640, 1630, 1620, 1600, 1590 (quinone, C=O, C=C), NMR (d_6-DMSO, ppm): 1.46 (3H, s, -Ċ-Me), 2.06 (3H, s, ϕ-Me), 5.98 (1H, s, ϕ-H), 12.77 (1H, chelated OH) and colorless tolypolides F_1 (IX) and F_2 (X). Tolyponone triacetate has no more OH group, thus VII has three OH groups. The UV-spectrum of a product obtained by the reductive acetylation of VII was closely similar to that of 4-keto-1,2,3,4-tetrahydrophenanthrene. These observations strongly suggested that VII would be represented by the part structure (A).

It was found that acid hydrolysis of Rifamycin S (3) under mild condition gave a compound which was identical with VII in its IR, NMR and mass spectra. Thus VII was established to be 2,8,12-trihydroxy-7,12-dimethyl-11,12-dihydro-furo-[3,2f]-1,4-naphthoquinone-11-one. When II was treated with 3% of concentrated hydrochloric acid in methanol, another quinone (VIII)*2, $C_{14}H_{11}NO_6$, was obtained in poor yield. Mild acid hydrolysis of VIII in an aqueous solution gave VII. Thus it was ascertained that the naphthoquinone moiety of II was the same as that of Rifamycin.



(A)



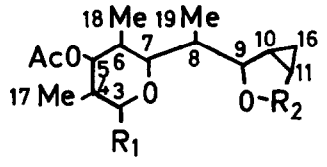
(VII) R = -OH

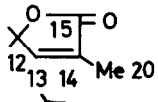
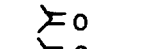
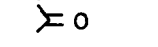
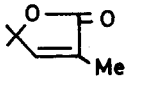
(VIII) R = $-NH_2$

Tolypolide F_2 (X), $C_{20}H_{28}O_6$, was found to be a deacetyl derivative of tolypolide F_1 (IX), $C_{22}H_{30}O_7$, UV λ_{max}^{EtOH} mμ (ε): 207 (8,800), IR ($CHCl_3$, cm^{-1}): 2730 (CHO), 1770 (γ-lactone), 1740 (-O-CO), 1725 (CO), 1665 (C=C), 960 (tetrahydrofuran ring), absence of OH, NMR ($CDCl_3$, ppm): 0.92 (6H, d, CH-Me), 1.05 (3H, d, CH-Me), 1.91 (3H, d, J=1.7, CH=C-Me), 2.08 (3H, s, OAc), 9.74 (1H, t,

*2 Recently, we obtained from Prof. V. Prelog the IR spectrum of the degradation product (4) of methylated Rifamycin S, which was identical with the spectrum of VIII.

-CH_2-C<u>H</u>O). These tolypolides were aldehydes and IX gave its semicarbazone, $C_{23}H_{33}N_3O_7$. Catalytic reduction of IX afforded dihydrotolypolide F_1 (XI), $C_{22}H_{32}O_7$, IR ($CHCl_3$, cm^{-1}): 2730, 1775, 1740, 1725, 960, absence of OH. Oxidation of IX with osmium tetraoxide followed by the oxidation with $Pb(OAc)_4$ gave a γ-lactone having aldehyde, tolypolide F_3 (XII), $C_{18}H_{26}O_6$, UV λ_{max}^{EtOH} mμ (ε): 206 (1,390), IR ($CHCl_3$, cm^{-1}): 2730, 1765, 1740, 1725, absence of OH, NMR ($CDCl_3$, ppm): 0.85, 0.92, 1.08 (each 3H, d, CH-<u>Me</u>), 2.08 (3H, s, O<u>Ac</u>), 9.75 (1H, t, CH_2-C<u>H</u>O). The chemical shifts and NMR spin-decoupling technique clearly demonstrated the part structure, from C_1 to C_9 in IX and XII, a group -CH=C- (Me) in IX and X and the hydrogenated group -CH_2-CH- (Me) in XI. A cyclopropane ring (0.42, 0.76, 1.20 and 1.77 ppm) was observed in IX and XI. Consideration of the unsaturation number of IX has led us to think of two additional rings, one of which was presumed to be a tetrahydrofuran ring from the IR spectrum, and the other should be a tetrahydropyran ring. On the basis of these findings, the following structures were proposed for tolypolides IX and XII. The data of the mass spectra of IX, XI and XII supported the proposed structures.



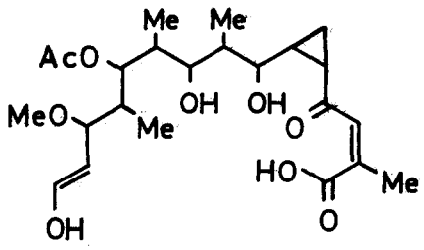
	R_1	R_2
(IX)	OHC-CH_2- (1 2)	
(XII)	OHC-CH_2-	
(XIII)	MeO-	
(XIV)	MeO-	

Another cleavage reaction of II was carried out with ozone to afford new γ-lactones, (XIII) and (XIV)*3. Tolypolide XIV has lost two carbon unit of C_1 and C_2 in IX. The acetal group in XIII suggested the position of the OMe group in II.

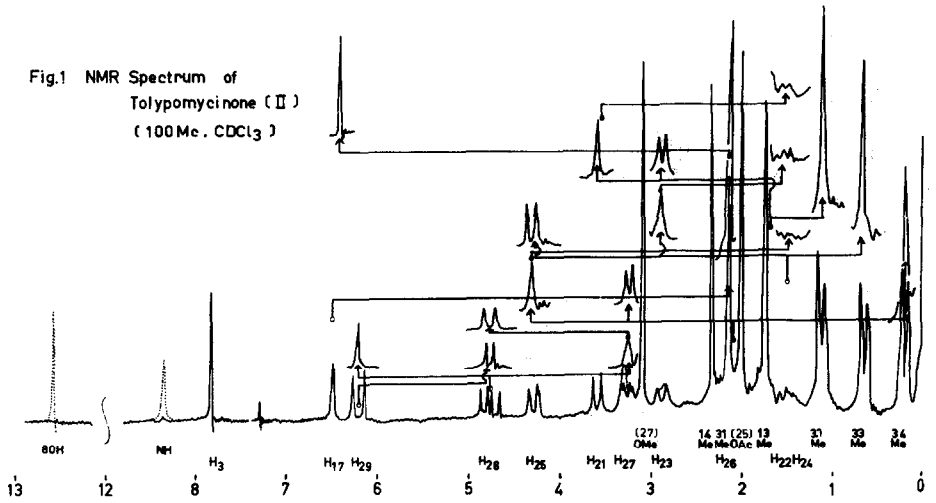
These findings led us to a conclusion that the part structure of II should be represented by an open chain structure (B). The molecular formula of II,

*3 A similar observation has been made with 1-buten-3-ol, which on ozonization gave acetic acid with concomitant loss of two carbon unit (5).

$C_{37}H_{43}NO_{13}$, can now be accounted for by the sum of the molecular formula for B, $C_{23}H_{36}O_9$, and that for VIII, $C_{14}H_{11}NO_6$, with a concomitant loss of 2 moles of H_2O. With tolypomycinone (II), the part structures from C_{21} to C_{29} and from C_{17} to C_{16}-Me were corroborated by the NMR spin-decoupling of II as shown in Fig. 1.



(B)

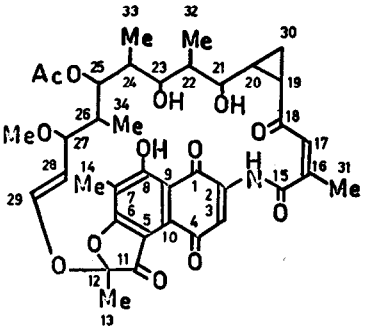


A fairly large value of the coupling constant, $J_{28, 29}$, demonstrates that the olefinic protons on C_{28} and C_{29} are trans. NOE[*4] studies with II showed that 31-methyl and proton at C_{17} are cis. Finally, II was assumed to have a carbonyl group on C_{18} by the following reasons: a γ-lactone is present in XII, the signal of the vinyl proton at C_{17} in II appears somewhat at low fields, IX has an α,β-unsaturated γ-lactone and the substraction of the UV absorption of VI from that for II remained a distinct absorption at 230 mμ (ε = 11,000) and 256 mμ (ε = 8,000), thus demonstrating the presence of a O=C-C=C-C=O group.

On the basis of these arguments the structure (II) is proposed for tolypomycinone. The NMR signals characteristic of the cyclopropane ring were not

*4 Nuclear Overhauser effect.

seen in the spectrum of II. However, methoxy tolypocyclonone (XV), $C_{38}H_{45}NO_{13}$ and ethoxy tolypocyclonone (XVI), $C_{39}H_{47}NO_{13}$ clearly exhibited the signals in high fields of the NMR spectra. They were obtained from II by treatment with 1% of concentrated hydrochloric acid in methanol or ethanol, respectively. Thus the protons of cyclopropane ring in II were assumed to shift in low fields due to the adjacent oxo group.



(II)

It should be mentioned that the structure (II)*5 corresponds to that of Rifamycin S with the methyl and the adjacent double bond replaced by the cyclopropane ring and the carbonyl, respectively. II has the same ansa-constitution as that of Rifamycins and Streptovaricins.

The authors wish to express their sincere thanks to Prof. Dr. V. Prelog of E.T.H., Zürich for the kind identification of his compound with VIII.

REFERENCES

1. T. Hasegawa, E. Higashide and M. Shibata, J. Antibiotics (Japan), in press.
T. Kishi, H. Yamana, M. Muroi, S. Harada, M. Asai and K. Mizuno, ibid. in press.
2. T. Kishi, S. Harada, M. Asai, M. Muroi and K. Mizuno, Tetrahedron Letters, in press.
3. P. Sensi, M. T. Timbal and G. Maffii, Experientia, 16, 412 (1960).
P. Sensi, R. Ballotta, A. M. Greco and G. G. Gallo, Farmaco Ed. Sci. 16, 165 (1961).
4. W. Oppolzer, V. Prelog and P. Sensi, Experientia, 20, 336 (1964).
5. P. S. Baily, Chem. Rev. 58, 925 (1958).

*5 The structure of tolypomycinone has recently been established by the X ray analysis with 8,21,23-tri-m-bromobenzoyl tolypomycinone by Kamiya et al. in this Division.