

ISOLATION AND STRUCTURES OF T-2636 ANTIBIOTICS

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Several antibiotics active against Gram-positive bacteria have been isolated from the culture broth of Streptomyces rochei var. bolubilis.^{*1} These antibiotics tentatively designated as T-2636 A, B, C, D and F, have been extracted from the filtered broth with ethyl acetate and finally separated by the chromatography on silica gel.

T-2636 A, ^{*2} (I), m.p. 207-210° (decomp.), $[\alpha]_D^{25} -235^\circ$ (c=1, EtOH), UV: $\lambda_{\max}^{\text{EtOH}}$
227 m μ ($\epsilon=46,900$), MW: 490 (V.P.O.), m/e 441 (MS, M⁺-60), C₂₇H₃₅N₀₈,
MIC^{*4}: 10-20.

T-2636 C, ^{*2,3} (II), m.p. 201-203° (decomp.), $[\alpha]_D^{25} -240^\circ$ (c=1, EtOH), UV: $\lambda_{\max}^{\text{EtOH}}$
227 m μ ($\epsilon=46,700$), MW: 439 (V.P.O.), m/e 459 (MS, M⁺), C₂₅H₃₃N₀₇, MIC:
0.5-0.75.

T-2636 D, (III), m.p. 190-191° (decomp.), $[\alpha]_D^{25} -226^\circ$ (c=1, EtOH), UV: $\lambda_{\max}^{\text{EtOH}}$
229 m μ ($\epsilon=48,000$), MW: 509 (V.P.O.), m/e 443 (MS, M⁺-60), C₂₇H₃₇N₀₈,
MIC: >100.

*1. It was isolated from a soil sample collected in Osaka.

*2. The structures proposed for T-2636 A and C in this paper are identical with those of Bundlin B and A, respectively. (M. Uramoto, N. Otake and H. Yonehara: Abstract paper of the Annual Meeting of Agricultural Chemical Society of Japan, page 141, 1969), published just before this communication.

*3. T-2636 C is identical with Lankacidin, a purified sample of which was kindly donated by CIBA in Basel.

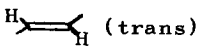
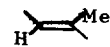
*4. Minimum Inhibitory Concentration against Staphylococcus aureus, ($\mu\text{g/ml}$).

T-2636 F, (IV), m.p. 178-179° (decomp.), $[\alpha]_D^{20} -210^\circ$ (c=1, DMF), UV: $\lambda_{\max}^{\text{EtOH}}$ 228 m μ ($\epsilon = 48,400$), MW: 503 (V.P.O.), m/e 443 (MS, M⁺-18), C₂₅H₃₅NO₇, MIC: 50-100.

T-2636 B, a neutral macrolide, m.p. 205-207°, $[\alpha]_D^{20} -92.4^\circ$ (c=1, EtOH), UV: $\lambda_{\max}^{\text{EtOH}}$ end absorption, MW: 851 (V.P.O.), m/e 850 (MS, M⁺), C₄₂H₇₄O₁₇, MIC: 50.

Acetylation of II afforded I, the 8-acetate (V), m.p. 203-204° (decomp.), C₂₇H₃₅NO₈ and the 8,14-diacetate (VI), m.p. 136-140° (decomp.), C₂₉H₃₇NO₉. Similarly, the acetylation of I gave VI and the acetylation of IV gave the 2',8,14-triacetate, (VII), m.p. 211-215° (decomp.), C₃₁H₄₁NO₁₀, which was identical with the 2',8-diacetate of III. Thus III is the 14-acetate of IV. Oxidation of III with CrO₃ gave I, while reduction of II with NaBH₄ led to IV. The reactions clearly demonstrated that III and IV were dihydro-I and-II, respectively. The above mentioned findings demonstrated that I, III and IV could be correlated with II.

The NMR and other spectral studies of the compounds suggested the presence of the following functional groups in II:

- (1) two secondary hydroxyls, from the NMR spectra of II and VI;
- (2) an acid amide group, from $\nu_{\text{NH-CO}}$ 3400 cm⁻¹ in VI and a signal at 8.0 ppm (d, 1H) which disappears with addition of D₂O;
- (3) two  (trans) and two  groups, from the fact that 4,6,10,12-octahydro-II-2'-ol 14-acetate (VIII, m.p. 172-175°, MS m/e 511 (M⁺), C₂₇H₄₅NO₈) was obtained by catalytic hydrogenation of III and that the NMR spectra of II, 6,12-tetrahydro-II (IX, m.p. 170-174°, MS m/e 463 (M⁺), C₂₅H₃₇NO₇) and VIII revealed the vinyl protons and allylic methyl groups corresponding to the structures;
- (4) a methyl ketone group, from a signal at 2.32 ppm (s, 3H) and $\nu_{\text{C=O}}$ 1725 cm⁻¹;
- (5) an isolated carbonyl group, from $\nu_{\text{C=O}}$ 1710 cm⁻¹ and the fact that UV spectrum of I was the same as that of II-2',18-diol 14-acetate (X, UV: $\lambda_{\max}^{\text{EtOH}}$ 231 m μ ($\epsilon = 49,600$), C₂₇H₃₉NO₈) which was obtained by NaBH₄ reduction of I;

- (6) a lactone group, from $\nu_{C=O}$ 1750 cm^{-1} and the fact that the potassium salt of II was obtained;
- (7) one each of tertiary methyl and secondary methyl, from a signal at 1.38 ppm (s, 3H) and a signal at 1.24 ppm (d, 3H, $J = 7$ cps); and
- (8) two methylene groups, from four protons at 2.2-2.5 ppm.

Mild acid hydrolysis of I yielded pyruvamide, MeCOCONH_2 , from water soluble fraction, m.p. 124-125°. On the other hand, lactamide, MeCH(OH)CONH_2 , was obtained from III by the same reaction, the latter being derived into its benzoate m.p. 94-101°, $[\alpha]_D^{+40}$ ($c=0.5$, EtOH).

On catalytic hydrogenation, II (IR $\nu_{\substack{\text{CHCl}_3 \\ \text{C=O}}} (\text{cm}^{-1})$ 1750, 1725, 1710) gave 6-dihydro-II (XI, $\text{C}_{25}\text{H}_{35}\text{NO}_7$, UV: $\lambda_{\text{max}}^{\text{EtOH}}$ 234 $\text{m}\mu$ ($\epsilon=21,600$), IR $\nu_{\substack{\text{CHCl}_3 \\ \text{C=O}}} (\text{cm}^{-1})$ 1740, 1725, 1700) and 6,12-tetrahydro-II (IX, UV: $\lambda_{\text{max}}^{\text{EtOH}}$ 245 $\text{m}\mu$ ($\epsilon=3,140$), IR $\nu_{\substack{\text{CHCl}_3 \\ \text{C=O}}} (\text{cm}^{-1})$ 1745, 1725, 1710). Subtraction of the UV absorption of XI from that of II leaves a distinct absorption maximum at 224 $\text{m}\mu$ ($\epsilon=35,600$). These absorption at 224 $\text{m}\mu$ and 234 $\text{m}\mu$ may be ascribable to an α,β -unsaturated lactone¹⁾ and a diene²⁾, or two dienes. In the IR spectra of II, XI and IX, no shift of carbonyl absorptions was observed, thus the presence of an α,β -unsaturated lactone group was ruled out.

The NMR spectrum of IX suggested the presence of two allylic methyl groups. The UV spectrum of IX ruled out the presence of a diene system.

Two single proton signals at 4.06 and 4.26 ppm in II, which are ascribable to secondary hydroxylic methine protons, showed upfield shift in the spectrum of IX. Thus the secondary hydroxyls should be present at positions α to double bonds. Mild oxidation of V with CrO_3 in pyridine gave 8-acetyl 14-oxo-II (XII, m.p. 197-200° (decomp.), $\text{C}_{27}\text{H}_{33}\text{NO}_8$, $\lambda_{\text{max}}^{\text{EtOH}}$ 233 $\text{m}\mu$ ($\epsilon=29,600$), 288 $\text{m}\mu$ ($\epsilon=16,000$)).

The presence of the following part structures, i.e., (A), (B) and (C), was corroborated by the NMR spin-decoupling of XII, which is shown in Fig. 1.

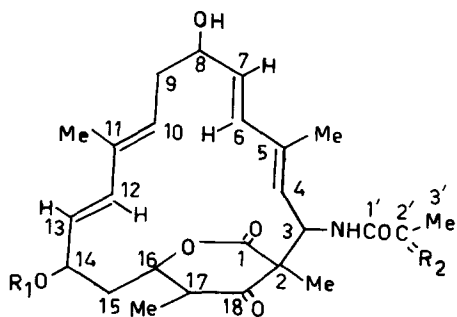
CrO_3 in pyridine. Methylene and methine protons adjacent to newly occurring carbonyl groups in XII and XIII showed a downfield shift as compared with those in II, thus they should be present at position α to the hydroxyl methines in II.

The methine proton at 2.56 ppm in VIII showed an upfield shift in 4,6,10,-12-octahydro-II-2',18-diol 14-acetate (XIV, m.p. 216-220°, MS m/e 513 (M^+), $\text{C}_{27}\text{H}_{47}\text{NO}_8$), which was obtained from X by catalytic hydrogenation, thus the isolated carbonyl group should be adjacent to this methine as shown in the part structure (C). The remaining part of the total molecule of XII can now be accounted for by a single tertiary methyl group (D).

The total structure of XII, therefore should be constructed by the part structures A, B, C and D. As XII has a diene and a dienone system²⁾, both of which contain an allylic methyl group, the only possible combination of the part structures to account for all these arguments has to be (b)-(c), (d)-(e), (f)-(i), (g)-(h) and (j)-(a).

On the basis of these findings, the structures (I), (II), (III) and (IV) have been proposed for T-2636 A, C, D and F.

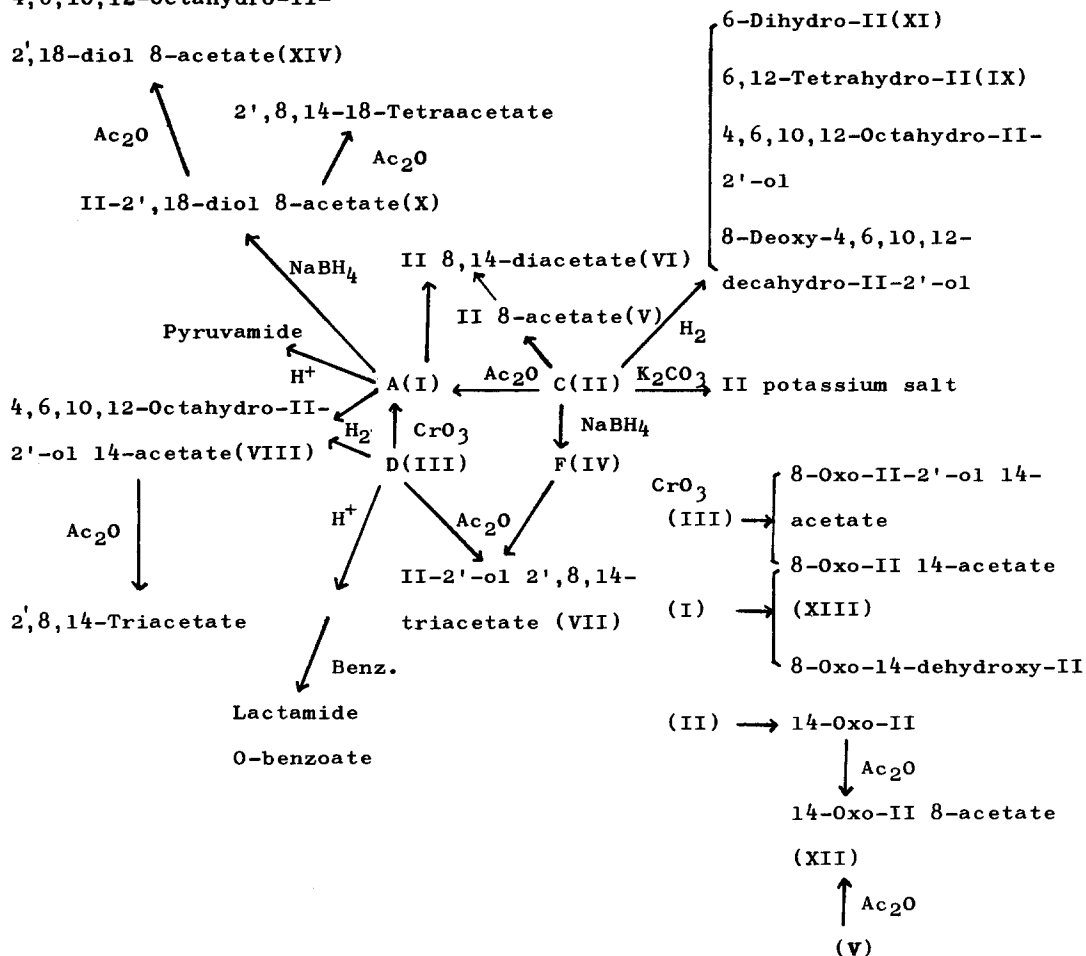
The absolute configuration of T-2636 A has been clarified as (2S, 3R, 8S, 14S, 16R, 17R) by the X-ray analysis of its 2'-p-bromophenylhydrazone in these laboratories.³⁾



| | R ₁ | R ₂ |
|----------------|----------------|----------------|
| T-2636 A (I) | -COMe | =O |
| T-2636 C (II) | -H | =O |
| T-2636 D (III) | -COMe | -H, -OH |
| T-2636 F (IV) | -H | -H, -OH |

4,6,10,12-Octahydro-II-

2',18-diol 8-acetate(XIV)



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