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THE CHEMISTRY OF THE LEUCOMYCINS. I.
PARTIAL STRUCTURE OF LEUCOMYCIN A_3

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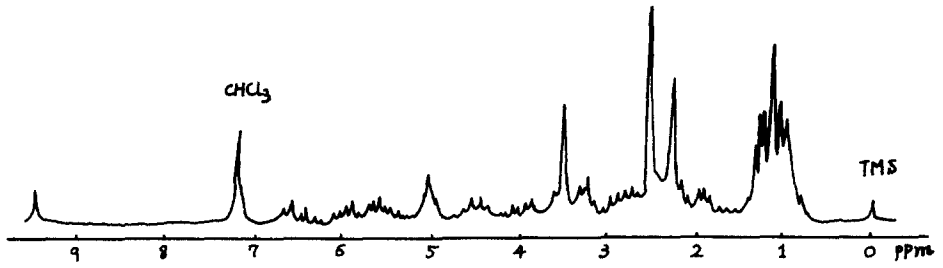
The leucomycins have been found to be a family of macrolide antibiotics isolated from Streptomyces kitasatoensis Hata by T. Hata et al[1]. In the course of earlier chemical studies, leucomycin A_1 proved to be an alcohol having a carbonyl group, a conjugated double bond and an epoxide[2)3)].

In this report, a new component named leucomycin A_3 is discussed, which is one of the most effective compounds and closely resembles leucomycin A_1. Leucomycin A_3 (I) could be separated from the leucomycin complex by chromatography on silicic acid, eluted by benzene-acetone, and crystallized from benzene as colorless prisms, m.p. 120-121°C, $[\alpha]_D$ -58.0° (c 2.0, MeOH), -55.4° (c 1.8)[***], λ_{max} 231.5 mμ ($E^{1\%}_{1cm}$ 351)[***], pka' 6.70.[***] Molecular weight determination by titration and osmometry (in chloroform) gave values of 835±10 and 850±25, respectively, and these values coupled with microanalytical data led to a molecular formula of $C_{42}H_{69}O_{15}N$ (Calcd. 827). It gives negative ninhydrin and Van Slyke nitrogen tests. The Tollen's and tetrazolium tests are positive. Zeisel determination shows the presence of one methoxyl group. The IR spectrum shows strong peaks at 1728 and 17[illegible] cm^{-1} (carbonyl), 1230 cm^{-1} (acetyl), and weak peaks at 2725 cm^{-1} (aldehyde) and 1661 cm^{-1} (double bond).

[***] Unless otherwise stated, rotations were measured in chloroform solution at 25°C, UV spectra were taken in methanol solution and pka' values were measured in 50% ethanol. Satisfactory analyses were obtained for all compounds for which molecular formulae are given.

Thr NMR spectrum in $CDCl_3$ suggests the presence of one dimethylamino group (2.49 ppm.), one methoxyl group (3.48 ppm.), one aldehyde group (9.65 ppm.), one acetyl group (2.22 ppm.), four olefinic protons (4H, 5.3-6.7 ppm.) and a number of C-methyl groups at around 1 ppm.

Diacetyl leucomycin A_3 (II) crystallizes as colorless needles, $C_{46}H_{73}O_{17}N$, m.p. 125-126°C, pka' 5.69. The IR spectrum of the acetate suggests the presence of a tertiary hydroxyl group in I, because II still retains a hydroxyl absorption.



NMR spectrum of leucomycin A_3 ($CDCl_3$: 100 Mc)

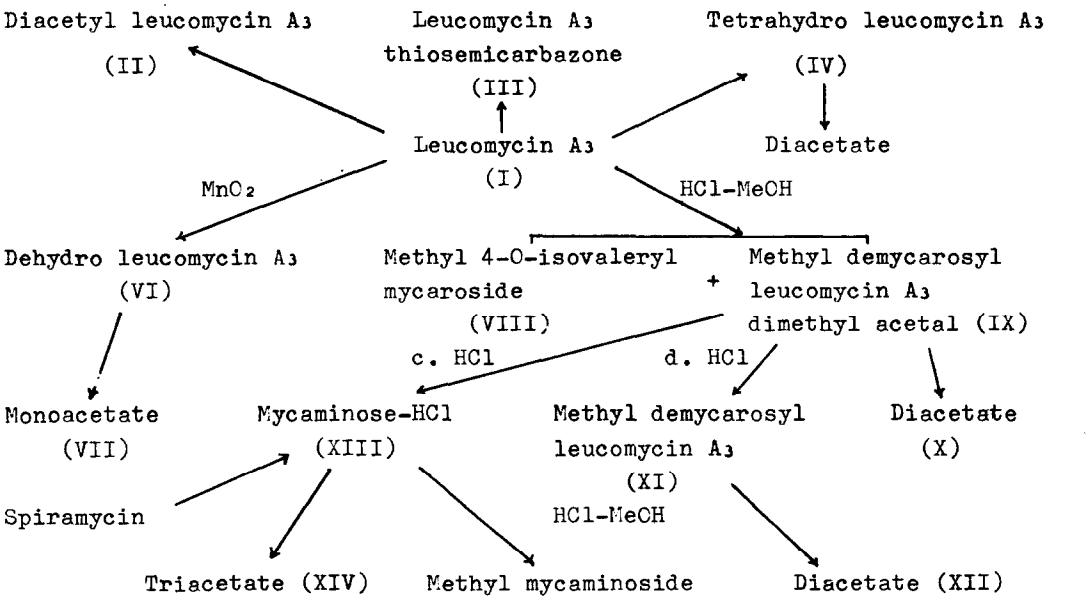
The thiosemicarbazone (III), $C_{43}H_{72}O_{14}N_4S$, m.p. 138-141°C, λ_{max} 232 mμ ($E^{1\%}_{1cm}$ 403) and 271.5 mμ ($E^{1\%}_{1cm}$ 270) shows a triplet at 7.62 ppm. (J 5 cps) in the NMR spectrum. This suggests the presence of a $-CH_2CHO$ group in I[4].

The olefinic nature of leucomycin A_3 is indicated by decolorization with permanganate and bromine, and is supported by catalytic hydrogenation (5% Pd-C) in ethanol (2 molar equivalents of hydrogen were absorbed in 2 hrs) to give the tetrahydroderivative (IV) in the form of white powder, $C_{42}H_{73}O_{15}N$, $[\alpha]_D$ -54.0° (c 1.3); diacetate (V), m.p. 115-118°C, $[\alpha]_D$ -74.0° (c 1.0).

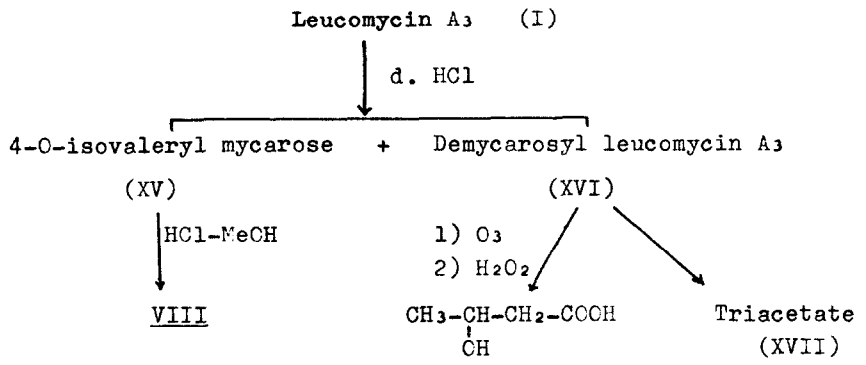
The UV spectrum of I, λ_{max} 231.5 mμ (ε 29100), which is similar to those of spiramycin[5] and its acid hydrolysis product, forocidine[5], λ_{max} 232 mμ (ε 29700), suggests the presence of a -CH=CH-CH=CH-CH-OR grouping. Oxidation of I with activated manganese dioxide produces dehydrcleucomycin A_3 (VI), $C_{42}H_{67}O_{15}N$, m.p. 140-141°C (dec.), $[\alpha]_D$ -34.0°, λ_{max} 224 mμ (ε 6200) and 279.5 mμ (ε 21900). This UV spectrum indicates an α,β,γ,δ-unsaturated ketone. From this reaction it becomes clear that I contains an α,β,γ,δ-un-

saturated alcohol group.

Alkaline hydrolysis of I gives the sodium salts of acetic acid and isovaleric acid (comparison with authentic samples by paper chromatography[6] and NMR spectra). Methanolysis of I in methanolic HCl, followed by silica gel chromatography of the liquid product affords α- and β-methyl 4-O-isovaleryl mycarosides (VIIIa and VIIIb), as identified by mass (M^+ 260), NMR and IR spectral comparisons with authentic samples obtained from leucomycin A1[2]. The remaining aqueous layer yields methyl demycarosyl leucomycin A3 dimethyl acetal (IX), $C_{33}H_{57}O_{12}N$, $[\alpha]_D$ -18.5° (c 1.45), λ_{max} 232 mμ (ε 28100), pka' 7.81. A dimethyl acetal group (3.15 and 3.25 ppm.) is observed in the NMR spectrum of IX (in $CDCl_3$), instead of an aldehydic proton. The aldehyde group is readily regenerated by dilute acid treatment, forming methyl demycarosyl leucomycin A3 (XI). This suggests that IX contains one methoxyl group in addition to an acetal group. Acetylation of IX affords

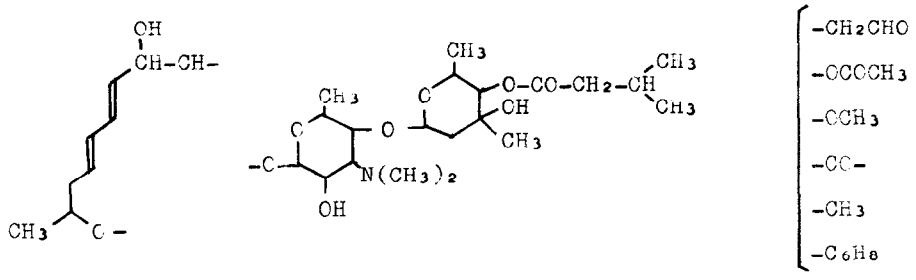


a diacetate (X), m.p. 179-181°C, $[\alpha]_D$ -10.5° (c 1.3), $C_{37}H_{62}O_{14}N$, pka' 5.40, having no hydroxyl group in the IR spectrum. That both IX and X have three $>CH-CH_3$ groups is evident from their NMR spectra in $CDCl_3$ and benzene.



Hydrolysis of IX with 6 N. HCl affords mycaminose hydrochloride (XIII), $C_8H_{17}O_4N.HCl.H_2O$, m.p. 113-116°C, which is identical with an authentic sample obtained from spiramycin[7].

I is hydrolyzed in dil. HCl to give 4-O-isovaleryl mycarose (XV) and demycarosyl leucomycin A3 (XVI). XV is methylated to VIII mentioned above. XVI crystallizes as needles, m.p. 199-202°C, $[\alpha]_D$ -14.0° (c 1.0), pka' 7.80. The molecular weight determination by titration gives a value of 607±10, which suggests the molecular formula $C_{30}H_{49}O_{11}N$. It forms a triacetate (XVII), $C_{36}H_{55}O_{14}N$, m.p. 195-196°C, pka' 5.35, M^+ 725 m/e. It can be concluded that the molecular weight of I should be 827. Upon oxidation of XVI with ozone followed by H_2O_2 treatment, β-hydroxy butyric acid is obtained. This suggests that I has the moiety $>C{=}CH{-}CH_2{-}\underset{\displaystyle CH_3}{\underset{|}{CH}}{-}C-$. On the basis



(A) (B) (C)

of the evidence given, partial formulae A, B and C incorporating fifteen oxygen atoms can be derived for leucomycin A3.

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