in thymectomized mice compared to normal mice at 1 h after infection.

In contrast to the results reported by TAKEYA et al.[6] with *Listeria monocytogenes* in vitro, we did not observe enhancement of intracellular killing of amastigotes in macrophages derived from thymectomized mice. These experiments establish that macrophage activity in experimental leishmaniasis is thymus independent. Such activity could be inherent to the macrophages or depend on immunologically competent tissues formed early in the ontogenic development of the host[17].

Resumen. Fagocitosis y destrucción de *Leishmania donovani* por macrófagos in vivo o in vitro es igual en macrofagos de ratones timectomizados e infectados que en macrófagos de ratones no timectomizados e infectados. Se concluye que la resistencia natural contra infección con *L. donovani* no es disminuida por la timectomía. Además, los datos indican que la actividad de los macrófagos es independiente del timo.

G. A. SCHMUNIS[18] and R. HERMAN

U.S. Department of Health, Education, and Welfare, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Parasitic Diseases, Bethesda (Maryland 20014, USA), 5 October 1970.

[17] M. L. TYAN, L. A. HERZENBERG and P. R. GIBBS, J. Immunol. *103*, 1283 (1969).

[18] Guest Worker, NIAID. Fellowship holder from the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Argentina and holder of award No. 5 F05 TW 1468-02 from the U.S. Department of Health, Education and Welfare, Public Health Service.

STUDIORUM PROGRESSUS

Structures of the Venturicidins A and B

The venturicidins A and B, two antifungal antibiotics from *Streptomyces aureofaciens*, strain Tü 342, are a 3-*O*-carbamyl-2-deoxy-D-rhamnoside and a 2-deoxy-D-rhamnoside, respectively, of an unknown aglycone[1]. Although the aglycone itself was decomposed during solvolytic reactions, spectroscopic coincidences strongly suggested the identity of the two aglycones. Additional evidence for this identity, the isolation of several common degradation products from the aglycone part, will be given in this paper.

The structure of venturicidin A was determined by restricted use of conventional organic chemistry methods, by an extensive application of NMR- and mass-spectrometry, and by a three-dimensional X-ray analysis of a heavy-atom derivative, the mono-*p*-iodobenzenesulfonate V.

X-ray analysis. The derivative V was prepared by treatment of venturicidin A with *p*-iodobenzenesulfonyl chloride in dry pyridine at room temperature. The crystals were grown from a water-acetone solution by slow cooling. Crystal data: $C_{47}H_{70}INO_{13}S \cdot H_2O$; *ortho*rhombic disphenoidal with $a = 5.76 \pm 0.05$, $b = 31.4 \pm 0.2$, $c = 31.3 \pm 0.2$ Å; $V = 5661$Å^3; $F(000) = 2168$. Space group $P2_12_12_1$ (D_4^2, No. 19), from systematic absences; $Z = 4$, $D_c = 1.21$ gcm^{-3}; molecular weight calc. 1034.03, found 1031. Mo Kα-radiation, λ taken as 0.7107 Å, $\mu = 7.3$ cm^{-1}. The limits of errors are given in the form of maximum errors.

The intensity measurements were made on a Siemens AED automatic four-circle diffractometer at room temperature. Because of the weak scattering of the crystals available, only 1147 independent reflections were recorded. The maximum value of $\sin \Theta/\lambda$ was 0.44, corresponding to a resolution of 0.7 Å. Absorption corrections were not applied, nor were extinction corrections.

The coordinates of the iodine atoms were first determined without ambiguity from a three-dimensional PATTERSON synthesis, sharpened on iodine, corresponding to an R value of 48%. The structure was then completely solved by a combination of FOURIER methods and block-diagonal least-squares calculations. The final refinements gave an R value of 10.1%[2].

Because of the poor resolution of the FOURIER syntheses, the following additional crystallographic evidence was used to determine unambiguously the nature of the atomic species and the positions of the double bonds: 1. local analysis of the variation of the thermal parameters, depending upon the choice of scattering curve for each atom; 2. stereochemical considerations such as valence angles and the approximate coplanarity of certain groups of atoms. In the figure a general projection of compound V is given, which shows that venturicidin A is a non-polyenic macrolide antibiotic.

The sugar moiety is bound to the aglycone through the oxygen atom on C-13, forming a β-glycosidic linkage. As no anomalous dispersion measurements were made, the correct absolute configuration, which is shown in the Figure, was deduced from the known configuration of D-rhamnose[3].

Because of the low resolution, in the FOURIER synthesis, of the side chain of the molecule, due to the large thermal motion as well as to the probable disorder of the final atoms of the chain, strict crystallographic evidence is lacking for the terminal methyl group (C-27). However, the following chemical evidence unambiguously demonstrates its presence.

Ozonolysis of Di-O-acetylventuricidin A. The ozonolysis was carried out in methylene chloride at −70 °C. Destruction of the ozonides with H_2O_2 and HCl in acetic acid, methylation by diazomethane, acetylation and column chromatography on silica gel yielded the following products:

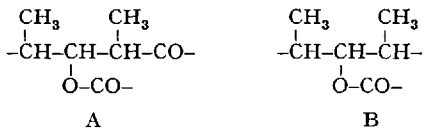
1. 1,4-di-*O*-acetyl-3-*O*-carbamyl-2-deoxy-D-rhamnose, identified by its NMR-spectrum.

[1] M. BRUFANI, W. KELLER-SCHIERLEIN, W. LÖFFLER, I. MANSPERGER and H. ZÄHNER, Helv. chim. Acta *51*, 1293 (1968).

[2] All the calculations were carried out on the Univac 1108 computer of the University, Rome, and were performed with the system of programs developed in Laboratorio di Strutturistica Chimica 'Giordano Giacomello' – CNR. The figure was produced by Johnson's ORTEP program.

[3] B. ISELIN and T. REICHSTEIN, Helv. chim. Acta *27*, 1146 (1944).

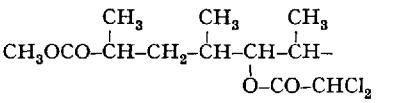
2. Compound VIII, $C_{23}H_{38}O_7Cl_2$, a non-distillable oil, ν_{max} 1735 cm^{-1}. The presence of 6 C-methyl groups (NMR-evidence) shows that this degradation product is derived from the region about C-15 through C-27. The NMR-spectrum shows further the presence of one *O*-methyl and one acetyl group. A singlet at 5.93 ppm (1 H) is assigned to the Cl_2CH–CO group. Two doublets of doublets at 5.03 and 4.77 ppm are due to hydrogens adjacent to ester oxygen atoms. The signal at 5.03 (J = 3.4 and 9.0 Hz) shows spin coupling with signals at 2.8 (CH–C=O) and 1.9 ppm, both of these again with signals in the C-methyl region (double resonance experiments). From this the partial formula A follows. Similar spin decouplings with the signal at 4.77 ppm show the presence of a group B.



A B

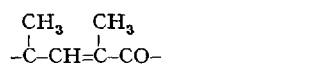
The mass spectrum shows a small molecular peak at *m/e* 496, the isotopic peaks indicating the presence of 2 chlorine atoms. Strong peaks at *m/e* 465 (M-31) and 436 (M-60) confirm the presence of an *O*-methyl and an acetyl group. A very strong signal at *m/e* 88 (McLAFFERTY rearrangement) indicates the presence of a methyl group in α position to a carbomethoxy group.

3. Compound IX, $C_{25}H_{42}O_9$, an indistillable liquid, ν_{max} 1735 cm^{-1}. The NMR-spectrum differs from that of compound VIII by the absence of the singlet at 5.93 ppm ($CHCl_2$–CO), by the presence of an additional OCH_3 signal and a singlet (2 H) at 3.41 ppm. The mass spectrum shows strong peaks at *m/e* 427 (M-59; $-COOCH_3$), 426 (M-60; AcOH); 395 (M-60-31) and 397 (M-89; –CO–CH_2–$COOCH_3$). On the basis of the spectral data, compound IX appears to be the analog of VIII, in which the dichloroacetic acid residue is replaced by the malonic acid methylester group. In support of this hypothesis, alkaline hydrolyses of VIII and IX, followed by diazomethane methylation and acetylation, gave the same compound X. Further the formation of a dichloroacetic acid ester can be interpreted as the substitution of the two malonic hydrogens by Cl_2 ($HCl + H_2O_2$ in the course of the ozonide decomposition) and the subsequent decarboxylation of the acid. Taking into account the relative positions of the lactonic oxygen and the double bond C-14–C-15 (from the X-ray structure), it is possible to establish the presence of the partial structure C in compound VIII.



C

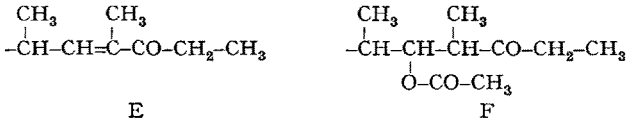
4. Compound XI, $C_{21}H_{34}O_5Cl_2$, λ_{max} 236 nm (log ε 4.00) in ethyl alcohol (α,β-unsaturated ketone); ν_{max} 1730 and 1665 cm^{-1}. The same compound can also be obtained by heating the acetate VIII with methanolic hydrogen chloride. The NMR-spectrum differs from that of compound VIII by the absence of the signal of the acetyl group, by the shift of the signal of one C-methyl to 1.78 ppm (singlet, 3 H), by the lack of the doublet of doublets at 5.03 ppm, and by the presence of a signal of an olefinic proton at 6.37 ppm (doublet, J = 9.2 Hz). The double resonance spectrum suggests the presence of the partial structure D.



D

The mass spectrum shows a strong molecular peak at *m/e* 436, the isotopic peaks confirming the presence of 2 chlorine atoms. A strong peak at *m/e* 308 (M-128) is interpretable by the loss of $CHCl_2COOH$, and a peak at *m/e* 407 (M-29) suggests that the substance is an ethyl ketone. Accordingly it is possible to write the partial

formula E for compound XI and consequently partial structure F for compound VIII.



E F

The sum of the two partial formulae, C and F, for compound VIII differs from its empirical formula by one CH_2 group, so that it is easy to write the complete molecular structures for compounds VIII and XI.

Alkaline degradation of venturicidin A. Alkaline degradation, carried out in aqueous 2 *N* sodium hydroxide under reflux, the distillation products being collected in a 2,4-dinitrophenylhydrazine solution, permitted the isolation of diethylketone as its crystalline 2,4-dinitrophenylhydrazone. The formation of diethylketone can be explained as a retroaldolic cleavage of the C-23–C-24 bond and confirms the presence of an ethyl ketone grouping and hence of the methyl group C-27, not detectable in the X-ray structure.

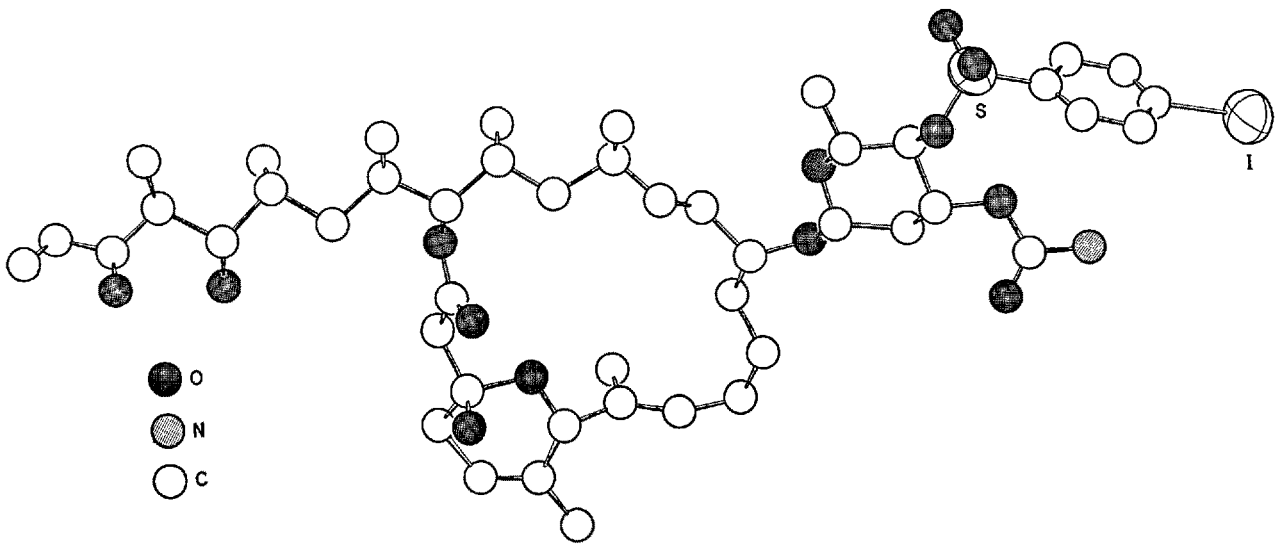
Ozonolysis and alkaline degradation, repeated on venturicidin B, gave the same products with the exception of 2-deoxy-D-rhamnose derivatives.

The presence of 2 methyl groups on olefinic carbon atoms could be confirmed by catalytic hydrogenation of venturicidin A, yielding a mixture of diastereometric hexahydrocompounds. The signals of 2 methyl groups originally present at 1.45 and 1.52 ppm are shifted to higher field in the hydrogenation product. Finally the occurrence of an OH IR-absorption band in the spectra of the acetates IV and VII confirms the tertiary hydroxyl group (pos. 3).

The structures of venturicidin A and B (I and II) and their respective acetates (III and IV) are not in agreement with the empirical formulae, $C_{43}H_{71}NO_{12}$ and $C_{42}H_{70}O_{11}$, previously deduced from elementary analyses and osmometric molecular weight determinations. Unfortunately the revised formulae, $C_{41}H_{67}NO_{11}$ and $C_{40}H_{66}O_{10}$, could not be confirmed by mass spectroscopy, no molecular ions being observable. However, diacetylventuricidin A (III)[1], $C_{45}H_{71}NO_{13}$, displays peaks at *m/e* 773 (M-60), 772 (M-61; $-NH_3$, $-CO_2$), 755 (M-60-18), 754 and 713 (M-2 × 60) in good agreement with the revised formulae.

Aglycone. The aglycone (VI) could not be obtained by usual solvolysis procedures because of its sensitivity to acid treatment, which is nicely understood on the basis of structure I. However, a periodate cleavage of the 3′-4′ bond in the sugar moiety of venturicidin B (II), followed by hydrolysis under extremely mild conditions, permitted the isolation of the aglycone VI, $C_{34}H_{56}O_7$, mp 135–137°. The NMR-spectra and double resonance spectra of VI and its diacetate VII are in good agreement with the structures indicated. The mass spectrum of the diacetate VII displays a very weak molecular ion with *m/e* 660 and stronger peaks at *m/e* 642 (M-18), 600 (M-60) and 582 (M-18-60), confirming the revised molecular formulae of the venturicidins.

Although the determination of the structure of the ozonolysis products requires that the structure of the macrolide ring is already known, the combination of chemical and X-ray evidence indicates that I and II are the structures of venturicidin A and B, respectively.



Riassunto. Con l'uso combinato di metodi chimici, spettroscopici, e di strutturistica chimica diffrattometrica è stato possibile assegnare le strutture I e II alle venturicidine A e B rispettivamente.

M. BRUFANI[4], S. CERRINI[5], W. FEDELI[5], C. MUSU[6], L. CELLAI[4] and W. KELLER-SCHIERLEIN[6]

Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi, I^a Cattedra, Roma (Italy), Laboratorio di Strutturistica Chimica 'Giordano Giacomello', CNR, Città Universitaria, Roma (Italy), and Organisch-chemisches Laboratorium der Eidg. Technischen Hochschule Zürich, CH-8006 Zürich (Switzerland), 18 December 1970.

[4] Istituto di Chimica Farmaceutica e Tossicologica dell'Università degli Studi, I^a Cattedra, Roma (Italy).

[5] Laboratorio di Strutturistica Chimica 'Giordano Giacomello' – CNR, Città Universitaria, Roma (Italy).

[6] Organisch-chemisches Laboratorium der Eidg. Technischen Hochschule, CH-8006 Zürich (Switzerland).