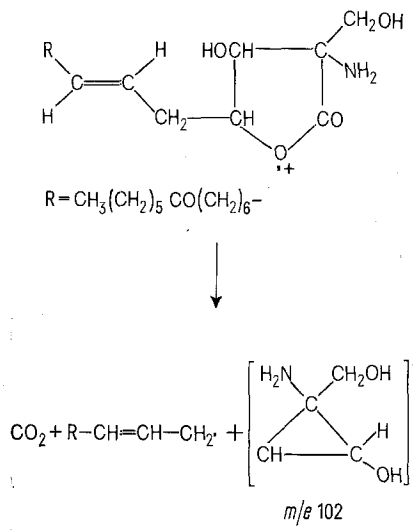
Structure of Thermozymocidin

For thermozymocidin, a new antifungal antibiotic from a thermophilic mould[1], $C_{21}H_{39}NO_6$, m.p. 170–172° (methanol), $[\alpha]_D^{24}$ + 4° (c = 1% dimethylsulfoxide), structure I (2-amino-2-hydroxymethyl-3,4-dihydroxy-14-oxoeicos-6-enoic acid) was established.

The IR-bands in nujol at 3375, 3240, 3150, 1710, 1665, 1605, 1572, 1533, 1412 and 965 cm^{-1} and the violet colour with ninhydrin indicated that it was an amino acid.

Hydrochloric acid in methanol transformed I into the aminolactone hydrochloride II, $C_{21}H_{38}NO_5Cl$, m.p. 158–164° (ethyl ether), which showed a rose colour with nihydrin and had IR-bands in nujol at 2855 (strong, $-NH_3^+$), 2500 (weak, $-NH_3^+$), and 1785 cm^{-1} (very strong, lactone) corresponding to the absorption pattern described by WEYGAND and MAYER[2] for some α-amino-γ-lactones. Since the characteristic fragmentation of such compounds[3] was also noted in the MS of II (base peak *m/e* 102) thermozymocidin had an α-amino-γ-hydroxy system in its molecule (Formulae).



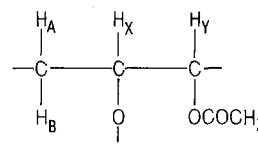
Hydrogenation of I with palladium on charcoal in methanol at room temperature and ambient pressure yielded a dihydroderivative III, $C_{21}H_{41}NO_6$, m.p. 166–168° (disappearance of the 965 cm^{-1} band), whereas acetylation of I with excess acetic anhydride in the presence of methanol gave the N-acetyl-γ-lactone IV, $C_{23}H_{39}NO_6$, m.p. 101–102° (ethanol-water), IR-bands (nujol) at 3510, 3300, 1760, 1710, 1650, 1565, and 965 cm^{-1}; NMR-signals (δ) at 6.57 (1H, b.s., –NHAc), 5.3–5.7 (2H, m, –CH=CH–), 4.5–4.7 (2H, m, –CHOH– and –CHO–), 3.86 (2H, s, $-CH_2OH$), 2.1 (3H, s, CH_3CONH–).

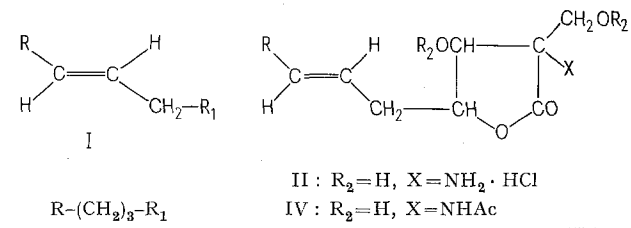
Acetylation of IV or I with acetic anhydride and pyridine produced the oily triacetyl-γ-lactone V, $C_{27}H_{43}NO_8$, IR-bands (nujol) at 1785, 1755, 1712, 1690, 1530 and 965 cm^{-1}, NMR-signals (δ) at 6.49 (1H, s, –NHAc), 5.75 (1H, d, J = 4.5 Hz, –CHOAc–), 5.3–5.7 (2H, m, –CH=CH–), 4.72 (1H, 6 lines, $-CH_2-CH$(O–)–CHOAc–), 4.5 (2H, s, $-CH_2OAc$), 2.01, 2.03 and 2.07 (3H, s, CH_3COO–).

This reaction and the comparison between the NMR-spectra of IV and I showed that the remaining two oxygen atoms of I consisted of a primary and a secondary alcohol.

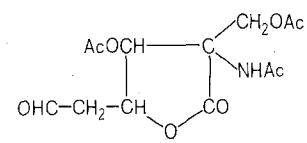
Osmium tetroxide oxidation of V in pyridine yielded a diol which, on sodium periodate treatment followed by chromatography on silica-gel, gave the two aldehydes VI, $C_{13}H_{17}NO_8$, m.p. 122–124°, and VII, $C_{14}H_{26}O_2$. Unstable VII was characterized as the corresponding acid VIII, $C_{14}H_{26}O_3$, m.p. 62–64° obtained through oxidation with alkaline silver oxide. Synthesis of VIII was accomplished by reaction of the cadmium Grignard reagent of 1-bromohexane and ω-carbethoxysuberoyl chloride[4].

The IR-spectrum of VI in $CHCl_3$ showed bands at 1795, 1760 and 1695 cm^{-1} and the NMR-spectrum signals (δ) at 9.78 (1H, t, J = 1 Hz, –CHO), 7.04 (1H, s, –NHAc), 4.88–5.16 (2H, m, –CHOAc and –CHO–), 4.43 (2H, s, $-CH_2OAc$), 2.88–3.14 (2H, m, $OHC-CH_2-CH$(O–)–), 2.02 (3H, s, CH_3COO–), 2.09 (6H, s, CH_3COO–). Double resonance experiments on compound VI showed that the aldehydic proton was adjacent to a methylenic group whose protons were also coupled with one of the hydrogens resonating at 4.88–5.16 δ. Complete elucidation of the structure of the polyfunctionalized part of the molecule of thermozymocidin was obtained by the observation, in the NMR-spectrum of V, of an ABXY system of the type:

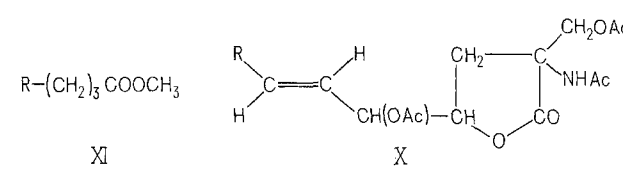


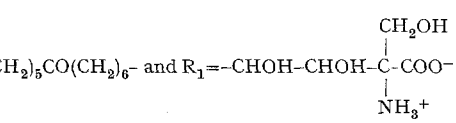


R-(CH2)3-R1
III



R–Y
VII: Y=CHO
VIII: Y=COOH
IX: Y=COOCH3





Where: R=$CH_3(CH_2)_5CO(CH_2)_6$– and R_1=–CHOH–CHOH–C(CH2OH)(NH3+)–COO–

[1] R. CRAVERI, P. L. MANACHINI and F. ARAGOZZINI, Experientia *28*, in press (1972).
[2] F. WEYGAND and F. MAYER, Chem. Ber. *101*, 2065 (1968).
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Here H_Y appeared as a doublet at 5.75 ($J = 4.5$ Hz) and H_X as 6 lines at 4.72 derived by the superposition of 2 triplets centered at 281 and 285.5 Hz, respectively, belonging to the X part of an ABX system where $J_{AX} = J_{BX} = 6.5$ Hz. Double resonance experiments confirmed this interpretation since irradiation at 2.48 δ transformed H_X and H_Y into 2 doublets with $J_{XY} = J_{YX} = 4.5$ Hz; irradiation at 4.72 δ reduced H_Y to a singlet at 5.75 δ and irradiation at 5.75 δ transformed H_X into a triplet at 4.72 δ with $J_{AX} + J_{BX} = 13$ Hz.

Chromic oxidation of dihydrothermozymocin III in acetic acid followed by methylation with diazomethane in ethyl ether of the reaction mixture, allowed the isolation of the ketoester XI, ruling out the alternative formulation X for compound V.

The relative configuration of the asymmetric carbon atoms of thermozymocidin could not be resolved by NMR-methods as it has been demonstrated[5] that stereoisomeric γ-lactones do not show any significant differentiation in the coupling of their protons. The strong absorption at 965 cm^{-1} in the IR-spectrum of I, II, IV and V suggested a trans configuration of the double bond.

Riassunto. La termozimocidina è un antibiotico antifungino prodotto da una muffa termofila. Le sue caratteristiche IR, NMR e MS, insieme a quelle dei suoi derivati e dei suoi prodotti di ossidazione, indicano la struttura I.

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12 January 1972.

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[6] The authors wish to thank Dr. G. SEVERINI RICCA for running the NMR-spectra and Dr. T. SALVATORI for the mass spectra.
[7] This research was supported by Società Italiana Resine S.I.R. (s.p.a.) Milano.

The Addition of Ethyl Azodicarboxylate to Pyrroles

Although the reactions of azodicarboxylic esters have been studied extensively[1–11] little is known of their addition to pyrroles[12] since only poorly characterised products were obtained in this study. In principle, the reaction of these esters with a pyrrole could proceed in three different ways: the azoester might attack the pyrrole nitrogen, a ring carbon atom or a side chain carbon atom. The present communication allows one to distinguish between these possible modes of attack.

It has been found that the free α- and β-positions in substituted pyrroles react with ethyl azodicarboxylate to give the corresponding dicarboethoxyhydrazino pyrrole. The conversion of these products into the corresponding benzene sulfonyl pyrrole hydrazines, in which the benzene sulfonyl hydrazine residue represent a blocking group of a free position which will not deactivate the ring, has not been realized.

The Table lists the various pyrroles used and the adducts formed[13]. In no case was a catalyst necessary. 2,4-Dimethyl (1) and 2,4-dimethyl-3-ethylpyrroles (2) react exothermally at room temperature in ether/*n*-pentane with ethyl azodicarboxylate at the α-position to give 1:1 adducts. 3-Methyl-4-ethylpyrrole (3) reacts at both α-positions to form a 2:1 adduct. 2,5-Dimethylpyrrole (4) reacts in one β position to give a 1:1 adduct. The above adducts are unstable and decompose on standing at room temperature. In contrast the adducts from the carbethoxypyrrole (5), and the acetylpyrrole (6) are stable at room temperature.

The structures assigned to the adducts are in all cases consistent with their NMR- and IR-spectra. Thus for example, the NMR-spectrum of (1) (Figure) in $CDCl_3$

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[13] All products gave satisfactory analytical data for C, H, N.