drolyzed by the cautious addition of 23 ml. of water, and the milky suspension was allowed to stand overnight. It was filtered and the filtrate, including ether washings, was distilled through a 6-cm. head packed with glass helices. The boiling ranges and indices of refraction of the amines agreed with earlier values.

We are grateful to Mr. E. F. Shelberg and staff of the Microchemical Department for the analyses reported here.

RESEARCH DIVISION
ABBOTT LABORATORIES
NORTH CHICAGO, ILLINOIS

Studies on Sarkomycin. Reaction with Diazomethane

WILLIAM B. WHEATLEY, CHARLES T. HOLDREGE, AND LOIS WALSH

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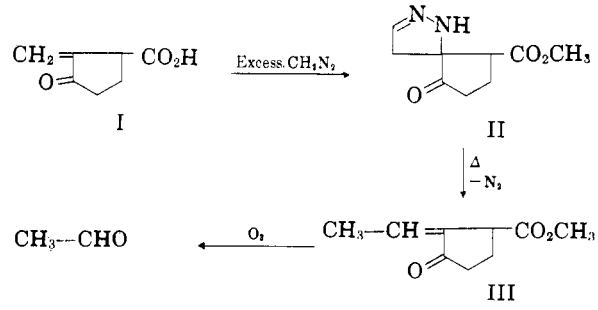
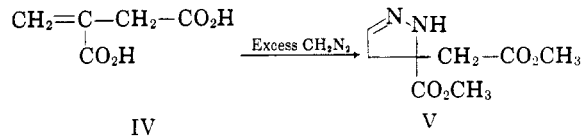
The active principle of sarkomycin, an antibiotic with suppressive action on the Ehrlich ascites tumor in mice, has been formulated as 2-methylene-3-oxocyclopentanecarboxylic acid, I.[1] We now wish to report additional experiments which are consistent with the proposed structure.

Treatment of sarkomycin with an excess of diazomethane gave a dark oil, the significant component of which appeared to be the pyrazoline ester, II. From this oil a crystalline hydrochloride was prepared, which gave an analysis in agreement with $C_9H_{12}N_2O_3 \cdot HCl$. On heating the oil to 110°, a sudden and rapid evolution of nitrogen occurred, following which an ester, III, could be distilled *in vacuo*. The analyses of this ester and its 2,4-dinitrophenylhydrazone indicated a formula of $C_9H_{12}O_3$ for III. An infrared peak at 6.1μ, similar to but sharper than that present in I[1] showed the presence of the carbon-carbon double bond. Ozonolysis of III yielded acetaldehyde, while ozonolysis of I yielded formaldehyde,[1] proving an over-all homologation at the methylene carbon.



Analogous additions of diazomethane to carbon-carbon bonds to give pyrazolines have been reported in several instances. For example, α-methylene-γ-phenyl-γ-butyrolactone has been shown to add diazomethane such that the carbon of the diazomethane becomes attached to the methylene carbon.[2] Aconic acid is first esterified, then converted to a pyrazoline by excess diazomethane.[3] This pyrazoline loses nitrogen to form the homolog of methyl aconate. We found that itaconic acid, IV, reacted with more diazomethane than was required for esterification of the two acid groups, giving a pyrazoline postulated as V by analogy with the foregoing facts. This pyrazoline did not lose nitrogen on heating up to 145°.



The authors are indebted to Dr. I. R. Hooper and his colleagues for supplying sarkomycin concentrates, and to Dr. L. C. Cheney for advice throughout this work.

EXPERIMENTAL[4]

Reaction of I with diazomethane. Fourteen liters of a Magnesol-treated methyl isobutyl ketone concentrate[1] containing a total of approximately 60 g. of sarkomycin acid were extracted with two 3.5-liter portions of water at *p*H 6.0. The aqueous extracts were concentrated slightly in a flash evaporator to remove dissolved methyl isobutyl ketone, then acidified to *p*H 3.0 with phosphoric acid. Extraction of the aqueous phase with one $^1/_5$-volume of ether followed by eight $^1/_{10}$-volumes of ether gave a total extract of about six liters, which was dried over sodium sulfate. The next day the ether solution was decanted and divided into two equal parts for esterification. Each portion was added dropwise to an ice-cold, stirred solution of diazomethane which had been prepared from 51.5 g. (0.5 mole) of N-nitrosomethylurea. After standing overnight while warming to room temperature, the two portions were combined and the solvent was distilled under reduced pressure. There remained 60 g. of dark, rather viscous oil, the crude pyrazoline ester.

Pyrazoline hydrochloride (II·HCl). The crude pyrazoline ester (2 g.) was dissolved in ether and excess dry hydrogen chloride was bubbled in. A red oil separated, from which 0.19 g. of crystalline material was obtained on disolution in *n*-butanol and dilution with ether. Several recrystallizations from methanol-ether gave pale orange crystals, m.p. 126–127° (gas evolution).

Anal. Calc'd for $C_9H_{12}N_2O_3 \cdot HCl$: C, 46.4; H, 5.6; N, 12.0; Cl, 15.2. Found: C, 46.4; H, 5.6; N, 12.5; Cl, 15.5.

Methyl 2-ethylidene-3-oxocyclopentanecarboxylate (III). Approximately 24 g. of crude pyrazoline ester was placed in a distilling flask and heated slowly to 110°. At this temperature, a vigorous evolution of nitrogen occurred. When this reaction had subsided, the material was vacuum-distilled. There was obtained 5.3 g. of III as a light yellow oil, b.p. 73–78° at 1.5 mm.

Anal. Calc'd for $C_9H_{12}O_3$: C, 64.3; H, 7.2. Found: C, 63.8; H, 7.3.

(1) Hooper, *et al.*, *Antibiotics and Chemotherapy*, **5**, 585 (1955).

(2) van Tamelen and Bach, *J. Am. Chem. Soc.*, **77**, 4683 (1955).

(3) Rekker, Brombacher, and Nauta, *Rec. trav. chim.*, **73**, 417 (1954).

(4) Melting points and boiling points are uncorrected. Analyses are by Mr. R. M. Downing; infrared data by Dr. F. M. Palermiti.

Comparison of the infrared spectrum of III with that of the best specimens of I showed an intensification of the 6.1 μ peak and a resolution of the broad 3.4 μ band of I into two peaks at 2.9 μ and 3.4 μ.

The crude pyrazoline ester used above had stood at room temperature for about two weeks; if pyrolysis and distillation were carried out immediately after reaction with diazomethane, the amount of distilled ester obtained could be increased to about 60% of the starting material. Apparently decomposition proceeds slowly on standing.

The *2,4-dinitrophenylhydrazone* of III melted at 139–141° after recrystallization from methanol.

Anal. Calc'd for $C_{15}H_{16}N_4O_6$: C, 51.7; H, 4.6; N, 16.1. Found: C, 51.7; H, 4.4; N, 16.6.

Ozonolysis of III.[5] A solution of 300 mg. of III in 10 ml. of ethyl acetate was ozonized at 0° until absorption of ozone ceased. The reaction mixture was heated on the steam-bath under reflux for 30 minutes with 10 ml. of water and 300 mg. of zinc dust. The cooled reaction mixture was filtered into a solution of 300 mg. of dimedone in 10 ml. of ethanol. Dilution with 20 ml. of water gave a two phase system; evaporation of the solvent from the upper layer left 68 mg. of solid, m.p. 138–141°. A mixture with authentic acetaldehyde-dimedone adduct showed no melting point depression. Heating both the ozonolysis product adduct and the authentic acetaldehyde adduct gave the same xanthene derivatives,[6] m.p. and mixture m.p. 174–176°.

Reaction of itaconic acid with diazomethane. Itaconic acid was added in small portions to an ice-cold ether solution of diazomethane prepared from 31 g. of N-nitrosomethylurea. When 8.2 g. had been added, the yellow color indicative of diazomethane disappeared. Evaporation of the solvent left an orange oil. Distillation up to 145° at 15 mm. gave 4.7 g. of distillate which distilled over a wide range. It partially crystallized and was assumed to contain some dimethyl itaconate; this was not investigated further. The residue from the distillation (4.4 g.) was taken up in 50 ml. of ether and filtered from 0.8 g. of insolubles. Treatment of the filtrate with dry hydrogen chloride yielded 3.0 g. of yellow crystals, which on recrystallization from isopropyl alcohol gave the hydrochloride of V, m.p. 145° (dec.).

Anal. Calc'd for $C_8H_{12}N_2O_4 \cdot HCl$: C, 40.6; H, 5.5. Found: C, 40.9; H, 5.7.

RESEARCH DIVISION
BRISTOL LABORATORIES, INC.
SYRACUSE 1, NEW YORK

(5) We are indebted to Drs. D. S. Tarbell and V. Boekelheide of the University of Rochester for their kindness in providing facilities and advice for this reaction.

(6) Horning and Horning, *J. Org. Chem.*, **11**, 95 (1946).