experiments which have allowed us to obtain sulfene at −196°, to observe its infrared spectrum at that temperature, and to follow certain of its reactions. Though the existence of sulfenes as short-lived intermediates has been demonstrated previously,[2,3] this is the first time that evidence has been obtained for a direct observation of any kind on any sulfene.

In the previous experiments[2] a "sandwich" technique was used. Modification of the apparatus has now been made to allow "homogeneous" trapping,[4] by which means, using methanol as the sulfene trap, the yield of methyl methanesulfonate was increased from 2.6 to 40%; at the same time the yield of methanesulfonyl chloride dropped from about 55% to a mere trace.[5] These experiments indicated that, as had been previously suspected,[2] the methanesulfonyl chloride formed in the "sandwich" experiments probably derived from trapping of the sulfene by hydrogen chloride, itself formed in the thermolysis. When hydrogen chloride was bled into the apparatus, a 58–59% yield of methanesulfonyl chloride was obtained. That this material was derived largely (and perhaps entirely) from sulfene was shown by using DCl instead of HCl as the sulfene trap and obtaining CH_2DSO_2Cl as the principal product.[6]

These experiments show that at least 50% of the chlorosulfonylacetic acid is converted to sulfene under these conditions. It therefore appeared worthwhile to examine the infrared spectrum of the thermolysate when trapped on a sodium chloride plate in a cryostat at −196°. The spectrum[7] (Figure 1a) shows bands ascribed to sulfene at 3170, 3040, 1330, 1230, and 950 cm^{-1}; the first two bands are assigned to the CH_2= group[8] and the second two appear not unreasonable for the $=SO_2$ function. On warming, these bands disappear and are simultaneously replaced by the spectrum of methanesulfonyl chloride (Figures 1b and 1c). This change, which begins around −140° and is complete by −80°, is clearly in full accord with the experiments with DCl described above.

When methanol is deposited (by the "homogeneous" technique[4]) along with the thermolysate, the infrared spectrum shows the characteristic bands at 1330 and 1230 cm^{-1}, the remainder being obscured by the methanol. On warming above −155° these bands begin to disappear and are replaced by the spectrum of methyl methanesulfonate, again, exactly as expected from the earlier experiments.

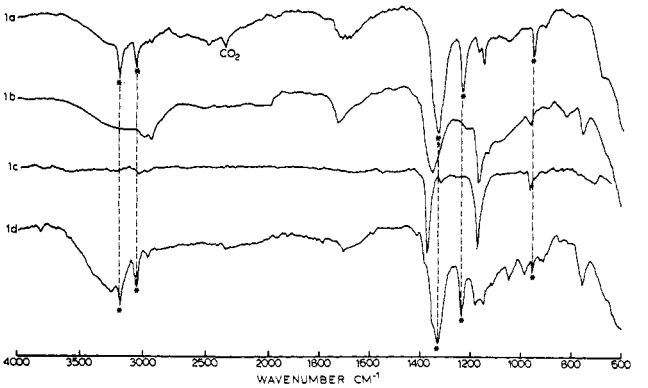


Figure 1. Infrared spectra of (a) the thermolysate from $ClSO_2CH_2COOH$ at −196°, (b) the same material as in (a) after warming to −70°, (c) authentic CH_3SO_2Cl, (d) the thermolysate from $CH_3SO_2OSO_2CH_3$ at −196°. The spectrum of authentic CH_3SO_2Cl was taken at room temperature in $CHCl_3$ solution (with a $CHCl_3$-filled NaCl cell in the reference beam); the other spectra are of the materials as collected on the NaCl plate (no solvent) with only air in the reference beam.

Final evidence that the unstable intermediate being observed in these reactions was indeed sulfene was obtained by thermolysis at 650° (<4 μ) of methanesulfonic anhydride.[9] The infrared spectrum of the thermolysate at −196° (Figure 1d) shows the same characteristic bands as those obtained by the other route.

Acknowledgment. This work was supported by the Petroleum Research Fund administered by the American Chemical Society. We wish to thank the donors of this fund.

(3) (a) For a summary of the earlier evidence see G. Opitz, *Angew. Chem., Int. Ed. Engl.*, **6**, 107 (1967); (b) J. F. King and T. W. S. Lee, *J. Amer. Chem. Soc.*, **91**, 6524 (1969).

(4) In the "homogeneous" technique the trapping reagent is bled into the apparatus at a point between the oven and the cold finger during the thermolysis, thereby giving an intimate mixture of trap and thermolysate on the cold finger.

(5) Yields were determined by distilling the material on the cold finger through a trap at −45° while allowing the cold finger to warm to room temperature. The excess methanol passed through the −45° trap while the methanesulfonyl chloride and methyl methanesulfonate were retained. Control experiments showed that methanesulfonyl chloride and methanol gave no methyl ester under these conditions.

(6) In two runs using DCl (isotopic purity 95%) the methanesulfonyl chloride showed the following composition as estimated by mass spectrometry: CH_3SO_2Cl, 12.6 and 19.9; CH_2DSO_2Cl, 87.4 and 80.1%. No detectable amount (<0.5%) of CHD_2SO_2Cl was present. Presumably at least some of the CH_3SO_2Cl arose from HCl produced in the thermolysis.

(7) In all spectra there was a variable background due to adventitious water and a few peaks presumably deriving from sulfene coupling products; in the previous work the formation of a small amount of presumed sulfene polymer was noted.[2]

(8) *Cf.* diazomethane, 3182 and 3069 cm^{-1} (N_2 matrix at 20°K) (C. B. Moore and G. C. Pimentel, *J. Chem. Phys.*, **38**, 2816 (1963)), and ketene, 3155 and 3062 cm^{-1} (Ar matrix, 20°K) (C. B. Moore and G. C. Pimentel, *ibid.*, **40**, 342 (1964)).

(9) M. H. Karger and Y. Mazur, *J. Org. Chem.*, **36**, 528 (1971). We thank Mr. D. R. K. Harding for calling this paper to our attention and Dr. E. G. Lewars for the specimen of methanesulfonic anhydride.

(10) Holder of National Research Council of Canada Scholarships, 1967–1971.

J. F. King,* R. A. Marty, P. de Mayo,* D. L. Verdun[10]
Department of Chemistry, University of Western Ontario
London, Canada
Received July 24, 1971

Negamycin, a Novel Hydrazide Antibiotic

Sir:

Negamycin is a new antibiotic isolated from the culture filtrate of three strains related to *Streptomyces purpeofuscus*, possessing a strong inhibitory activity against resistant Gram-negative bacteria including *Pseudomonas*.[1] We report herein the structural elucidation and partial synthesis of negamycin, and an interesting novel acid-catalyzed rearrangement of 1-methylhydrazinoacetic acid discovered during the structural study.

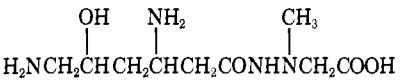
Negamycin (**1**) has the formula $C_9H_{20}N_4O_4$ (derived from the high-resolution mass spectrum of di-*N*-acetylnegamycin methyl ester (**2**), mp 157–158°; M^+ = 346.188, calcd for $C_{14}H_{26}O_6N_4$, M^+ = 346.185) and shows: mp 110–120° dec; $[\alpha]^{29}D$ +2.5° (*c* 2, H_2O); pK_a' = 3.55, 8.10, and 9.75; uv end absorption; ir (KBr) 3430, 3200, 3050, 2950, 1660, 1590, 1405, 1320,

(1) M. Hamada, T. Takeuchi, S. Kondo, Y. Ikeda, H. Naganawa, K. Maeda, Y. Okami, and H. Umezawa, *J. Antibiot.*, 23, 170 (1970).

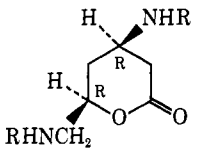
1140, 1050, 970, 890, 820, 720 cm^{-1}; ninhydrin positive, Rydon-Smith positive, red tetrazolium positive, and Sakaguchi negative. The nmr (D_2O) of **1** shows a methyl group (δ 3.42, singlet), an isolated methylene (δ 4.18, singlet), and the presence of a partial structure $CH_2CHCH_2CHCH_2$ confirmed by the application of double resonance technique.

A novel amino acid was isolated in good yield by acid hydrolysis (6 *N* HCl, reflux for 1 hr), accompanied by sarcosine (**3**) and methylamine (**4**). Nmr analysis showed that this amino acid contains the partial structure described above, and that it is δ-hydroxy-β-lysine (3,6-diamino-5-hydroxyhexanoic acid) (**5**) proven by conversion to be the di-*N*-acetyl derivative of δ-lactone (**6**) (mp 190–192°; $[\alpha]^{26}D$ −7.3° (*c* 2.7, H_2O); ORD positive Cotton effect). The structure of the lactone was unambiguously confirmed to be *cis*-4-amino-6-(aminomethyl)tetrahydro-2*H*-pyran-2-one by nmr analysis. Mild acid hydrolysis of negamycin (1 *N* HCl, reflux for 3 hr) afforded 1,2-dimethylhydrazine (**7**) in excellent yield and 1-methylhydrazinoacetic acid[2] (**8**), besides **4** and **5**. The experimental evidence indicates that **8** is converted into **7** along with **3** and **4** by mild acid hydrolysis, suggestive of a new rearrangement of the hydrazinoacetic acid, the original component of **1**, during acid hydrolysis.

Although there are three possible CO–NH bonds in a dipeptide of **5** and **8**, the hydrazide structure of negamycin was unambiguously determined by the comparison of the high-resolution mass spectrum of **2** with those of di-*N*-acetyl-d_6-negamycin methyl-d_3 ester and di-*N*-acetyl-d_6-negamycin methyl ester.



1



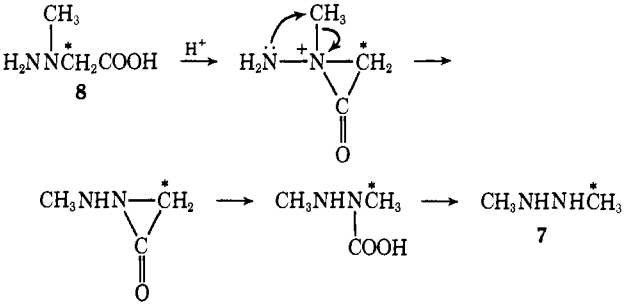
6, R = Ac

With regard to the absolute configuration of the amino acid moiety of **1**, the application of Hudson's lactone rule[3] and Klyne's lactone sector rule[4] suggests assignment of the L_G configuration to the asymmetric carbon of the δ position of the lactone **6**. This has been confirmed by a synthesis of the enantiomer from a carbohydrate precursor of known configuration as described below. Therefore, the absolute structures of δ-hydroxy-β-lysine (**5**) and negamycin (**1**) are (3*R*,5*R*)-3,6-diamino-5-hydroxyhexanoic acid and [2-{(3*R*,5*R*)-3,6-diamino-5-hydroxyhexanoyl}-1-methylhydrazino]-acetic acid, respectively.

Furthermore, the structure of **1** has been confirmed by a partial synthesis, by treating the *N*-hydroxysuccinimide ester of di-*N*-benzyloxycarbonyl-δ-*O*-tetrahydropyranyl-β-lysine with 1-methylhydrazinoacetic acid (**8**) followed by removal of the protecting groups with HBr–AcOH to give synthetic negamycin in 40% yield.[5] The protection of the δ-hydroxy group of amino acid with dihydropyran was essential for the peptide synthesis.[6]

The conversion of 1-methylhydrazinoacetic acid (**8**) into 1,2-dimethylhydrazine (**7**) seems to be a novel rearrangement. For a mechanistic study, 1-methylhydrazino[α-^{14}C]acetic acid (3,857,400 cpm/mmol) was prepared according to the method of Carmi, *et al.*,[2] and hydrolyzed with 1 *N* HCl to afford 1,2-dimethylhydrazine (3,597,700 cpm/mmol, 93% specific activity) as a main product. This evidence suggests strongly that the reaction is an intramolecular rearrangement, and a tentative mechanism is proposed in Scheme I.[7]

Scheme I



Finally, we became interested in the stereochemical correlation and the biological activity of the antipode of **5**, which may be derived from 3-amino-3-deoxy-D-glucose easily available by fermentation of *Bacillus aminoglycosidicus*.[8] (3*S*,5*S*)-3,6-Diamino-5-hydroxyhexanoic acid was prepared from 3-amino-3-deoxy-D-glucopyranose,[9] proving the correctness of the structure, including the absolute configuration of the amino acid moiety of **1**, since the absolute structure of the starting material is known.[8] The biological activity of the hydrazide derivative will be described in due course.

Acknowledgment. We wish to express our deep gratitude to Dr. M. Sezaki and Miss Y. Ikeda for their technical assistance.

(2) The hydrazino acid was confirmed to be 1-methylhydrazinoacetic acid identical with an authentic sample; A. Carmi, G. Pollack, and H. Yellin, *J. Org. Chem.*, 25, 44 (1960).

(3) C. S. Hudson, *J. Amer. Chem. Soc.*, 32, 338 (1910); B. Witkop, *Experientia*, 12, 372 (1956).

(4) J. P. Jennings, W. Klyne, and P. M. Scopes, *J. Chem. Soc.*, 7211 (1965).

(5) The parent δ-hydroxy-β-lysine was obtained from natural negamycin, and the synthetic negamycin was confirmed to be identical with the natural one in all respects including biological activity.

(6) The synthesis of a δ-hydroxy amino acid peptide was unsuccessful at the final stage because of the presence of the free δ-hydroxy group. See, for instance, H. Zahn and L. Zürn, *Justus Liebigs Ann. Chem.*, 613, 76 (1956).

(7) Sarcosine, 1,2 dimethylhydrazine, 1,1-dimethylhyrazine, and methylhydrazine are all stable under the reaction condition, and 2-methylhydrazinoacetic acid and 1-ethoxycarbonyl-2,2-dimethylhydrazine, possible candidates for the intermediate, afforded methylamine and glycine and 1,1-dimethylhydrazine, respectively, under the reaction conditions. We are indebted to Professor J. C. Sheehan for the discussion on the decomposition mechanism of α-lactams.

(8) S. Umezawa, K. Umino, S. Shibahara, M. Hamada, and S. Omoto, *J. Antibiot., Ser. A*, 20, 355 (1967).

(9) A detailed account of the transformation will be published in the near future.

Shinichi Kondo,* Seiji Shibahara, Shuji Takahashi
Kenji Maeda, Hamao Umezawa
Institute of Microbial Chemistry
Shinagawa-ku, Tokyo, Japan

Masaji Ohno
Basic Research Laboratories, Toray Industries, Inc.
Kamakura, Japan

Received June 29, 1971