The granular solid after filtration, washing with water and drying *in vacuo* was recrystallized from 8 ml. of 50% aqueous ethanol giving 1.1 g. of colorless needles melting at 214–215°. Further recrystallizations raised the melting point to 216–217°. The crystalline product had maximum a values of 17.5 and 26.8 at λ 239 and 307 mμ, respectively, in 0.1 N hydrochloric acid.

Anal. Calcd. for $C_{24}H_{36}N_4O_9$: C, 54.95; H, 6.92; N, 10.68. Found: C, 55.09; H, 7.06; N, 10.62.

N^6-O-Dipropionylcytosamine.—Cytosamine (0.40 g.) was dissolved in 1.5 ml. of warm propionic anhydride and the mixture allowed to stand overnight. After removing excess reagent *in vacuo*, water was added and the solution lyophilized. The white product was suspended in 5 ml. of water and the pH adjusted to 8.0 with alkali. A granular precipitate weighing 0.40 g. was obtained on filtration, washing with water and drying *in vacuo*. Recrystallization from 3 ml. of 33% aqueous ethanol gave 0.28 g. of colorless needles melting at 124–128°. The product had maximum a values of 16.4 and 27.5 at λ 238 and 307 mμ, respectively, in 0.1 N hydrochloric acid. Microanalytical data suggest a non-homogeneous product.

Anal. Calcd. for $C_{24}H_{38}N_4O_8$: C, 56.45; H, 7.50; N, 10.97. Found: C, 55.09; H, 7.56; N, 10.89.

Action of Diazomethane on N^6-(p-Nitrobenzoyl)-cytosamine.—To N^6-(p-nitrobenzoyl)-cytosamine (0.41 g.) dissolved in 25 ml. of absolute ethanol was added a solution of 0.5 g. of diazomethane in 25 ml. of ether. The solution after standing at room temperature overnight was filtered and concentrated to dryness *in vacuo*. The orange gum was dissolved in methylene chloride and precipitated as a red oil by the addition of n-heptane. Upon drying *in vacuo* an amorphous yellow powder was obtained which had no microbiological activity when tested against our *E. coli* test organism. The above powder, which was combined with a second crop, obtained by evaporating the above mother liquors, was dissolved in aqueous methanol and the pH adjusted to 3.3 with hydrochloric acid. Lyophilization afforded 0.30 g. of yellow-orange powder which on dissolving in ethanol and precipitating with acetone gave 0.1 g. of hygroscopic orange powder. The product had maximum a values of 17.6 and 15.4 at λ 274 and 320 mμ, respectively, in 0.1 N hydrochloric acid and showed but one ionizable group (pK_a' 7.0) by potentiometric titration.

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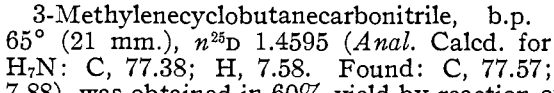
DETROIT 32, MICHIGAN

COMMUNICATIONS TO THE EDITOR

SYNTHESIS OF SUBSTITUTED ALKYLIDENECYCLOBUTANES

Sir:

Fluorine-containing cyclobutanes are readily prepared by cyclo-addition of tetrafluoroethylene, chlorotrifluoroethylene and 1,1-dichloro-2,2-difluoroethylene to olefins,[1] but no widely applicable direct synthesis of non-fluorinated cyclobutanes has been described. Recently Alder and Ackermann[2] have reported the synthesis of 3-alkylidene-1,2-cyclobutanedicarboxylic anhydrides by reaction of allenes with maleic anhydride. We have found independently that cyclo-addition of allenes to appropriately substituted olefins affords a general route to substituted 3-alkylidenecyclobutanes.



A = CN, CO_2R, CO_2H, CHO, Aryl

3-Methylenecyclobutanecarbonitrile, b.p. 64–65° (21 mm.), $n^{25}D$ 1.4595 (*Anal.* Calcd. for C_6H_7N: C, 77.38; H, 7.58. Found: C, 77.57; H, 7.88), was obtained in 60% yield by reaction of allene with a large excess of acrylonitrile under autogenous pressure at 175–225°. The structure of this cyclobutane was established by proton magnetic resonance and infrared spectra and by conversion to 3-methylcyclobutanecarboxylic acid, the anilide of which melted at 127–128° in agreement with the value reported by Kazanskii and Lukina.[3] The 2,6-(and/or 2,7)-dicyano-1,2,3,4,5,6,7,8-octahydronaphthalene, m.p. 143.5-144.5° (*Anal.* Calcd. for $C_{12}H_{14}N_2$: C, 77.38; H, 7.58; N, 15.04; mol. wt., 186. Found: C, 77.27; H, 7.50; N, 15.09; mol. wt., 175) was obtained as a secondary product in 15–20% yield. When equimolar amounts of acrylonitrile and allene were used, the octahydronaphthalene was the major product and only 15–20% yields of the cyclobutane were obtained. Substituted methylenecyclobutanes also have been obtained by reaction of allene with methyl acrylate, methacrylonitrile, acrylic acid, methyl methacrylate, methacrolein, α-acetoxyacrylonitrile, styrene, α-methylstyrene, 1,1-diphenylethylene, 4-vinylpyridine and indene. In many of these cases the corresponding octahydronaphthalenes were also isolated and identified.

Mixtures of isomeric cyclobutanes have been obtained from substituted allenes. 1,1-Dimethylallene and acrylonitrile gave 2,2-dimethyl-3-methylenecyclobutanecarbonitrile, b.p. 111° (100 mm.), $n^{25}D$ 1.4503 (*Anal.* Calcd. for $C_8H_{11}N$: C, 79.27; H, 9.15; N, 11.58. Found: C, 79.24; H, 9.26; N, 11.45) and 3-isopropylidenecyclobutanecarbonitrile, b.p. 138° (100 mm.), $n^{25}D$ 1.4691 (*Anal.* Found: C, 78.99; H, 9.11; N, 11.76) in a combined yield of 66%. Cyclobutanes have also been obtained from other substituted allenes

(1) D. D. Coffman, P. L. Barrick, R. D. Cramer and M. S. Raasch, THIS JOURNAL, **71**, 490 (1949).

(2) K. Alder and O. Ackermann, *Chem. Ber.*, **90**, 1697 (1957).

(3) B. A. Kazanskii and M. Yu. Lukina, *Izvest. Akad. Nauk, SSSR, Otdel. Khim. Nauk*, 47 (1951); *C. A.*, **46**, 4491 (1952); **45**, 2878 (1951).

including diethyl 2,3-butadienylmalonate and 1-acetoxy-2,3-butadiene.

A more complete description of the synthesis of cyclobutanes from allenes and substituted olefins and the conversion of alkylidenecyclobutanes to cyclobutenes, alkylidenecyclobutenes and other dienes will be published shortly.

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KANAMYCIN. I. CHARACTERIZATION AND ACID HYDROLYSIS STUDIES

Sir:

Kanamycin, discovered by Umezawa and co-workers,[1] is a water-soluble basic antibiotic active on Mycobacteria, Gram-positive and Gram-negative organisms. It has been characterized as the base, the sulfate and other derivatives.[2]

Kanamycin is isolated from fermentation broths by adsorption on Amberlite IRC-50[3] resin in the sodium cycle and elution with aqueous hydrochloric acid. The eluate is neutralized, diluted and readsorbed on IRC-50 which has been regenerated with ammonium hydroxide. The column is eluted with 0.2 *N* NH_4OH, the eluate concentrated *in vacuo* to approximately 50–100 mg./ml. of kanamycin activity, diluted with 0.8–1 volume of methanol and adjusted with H_2SO_4 to *p*H 8.0–8.2. Kanamycin sulfate crystallizes slowly in small irregular pale yellow prismatic crystals.

The crystalline kanamycin sulfate may be purified by repeated recrystallization from methanol–water at *p*H 7.8–8.2 to give white irregular prismatic crystals. This preparation contains adsorbed moisture which is removed with difficulty. Samples for analytical determinations were dried to constant weight at 170° *in vacuo*. *Anal.* Calcd. for $C_{18}H_{36}N_4O_{11}\cdot H_2SO_4$: C, 37.13; H, 6.58; N, 9.62; S, 5.50; $SO_4^=$, 16.6; neut. eq., 145.6; mol. wt., 600.6 (monohydrate). Found: C, 37.3, 37.4, 37.3; H, 6.8, 6.6, 6.3; N, 9.3, 9.6; S, 5.5; $SO_4^=$, 16.8; neut. eq., 146.6; mol. wt. 604 (X-ray on undried material).[4] The formula containing two less hydrogen atoms, although improbable, is not excluded by analyses on this and other derivatives.

The sulfate is soluble in water, insoluble in the common alcohols and non-polar solvents. It shows no melting point, decomposing over a wide range above 250°. It was converted to kanamycin base by treatment of an aqueous solution with a strongly basic anion-exchange resin, concentration and crystallization with methanol–ethanol mixture, $[\alpha]^{24}D$ + 146 (*c* 1, 0.1 *N*, H_2SO_4). *Anal.* Calcd. for $C_{18}H_{36}N_4O_{11}$: C, 44.6; H, 7.5; N, 11.6; neut. eq., 121.1; mol. wt., 484.5. Found: C, 44.7, 45.0; H, 7.40, 7.6; N, 11.0, 11.5 (Dumas); 11.8, 11.8 (Van Slyke); neut. eq., 121.5; mol. wt. 468, 444 (Rast); 427, 490 (Signer). Kanamycin gives positive Molisch, ninhydrin and Elson–Morgan tests and negative reducing sugar, Sakaguchi and maltol tests. Treatment with 40% sulfuric acid for 100 min. at 100° yields a product with an ultraviolet spectrum identical to that obtained from a pentose under similar conditions.

Treatment of kanamycin base with acetic anhydride and methanol yields tetra-N-acetylkanamycin[2b] which was recrystallized from aqueous methanol, m.p. 250–255° dec. *Anal.* Calcd. for $C_{26}H_{44}N_4O_{15}$: C, 47.9; H, 6.8; N, 8.4. Found: C, 48.5; H, 6.90; N, 8.3. Kanamycin picrate was prepared by treating kanamycin base in water with picric acid. The product was recrystallized from boiling water, m.p. 225–230° with decomposition. *Anal.* Calcd. for $C_{42}H_{48}N_{16}O_{39}$: C, 36.0; H, 3.46. Found: C, 36.2, 36.2; H, 3.62, 3.60. Kanamycin also has been characterized by the formation of a series of Schiff bases. Treatment of kanamycin base in water with *p*-chlorobenzaldehyde in isopropyl alcohol yielded tetra-N-*p*-chlorobenzylidene kanamycin, m.p. 213–216° with decomposition. *Anal.* Calcd. for $C_{46}H_{48}Cl_4N_4O_{11}$: C, 56.7; H, 4.96; N, 5.75; mol. wt., 974. Found: C, 56.94, 56.74; H, 5.02, 4.73; N, 5.94, 5.73; mol. wt. 971 (Signer). In a similar fashion we have prepared the tetra-N-veratrylidene kanamycin, m.p. 173–175°. *Anal.* Calcd. for $C_{54}H_{68}N_4O_{19}$: C, 60.22; H, 6.32; N, 5.20. Found: C, 60.35; H, 6.28; N, 5.14. Tetra-N-salicylidene kanamycin melted at 272–274° with decomposition. *Anal.* Calcd. for $C_{46}H_{52}N_4O_{15}$: C, 61.33; H, 5.77. Found: C, 61.25; H, 5.75. Tetra-N-*p*-methoxybenzylidene kanamycin melted at 193–196° with decomposition. *Anal.* Calcd. for $C_{50}H_{60}N_4O_{15}$: C, 62.7; H, 6.27. Found: C, 62.6; H, 6.66.

Paper chromatography of kanamycin preparations revealed a second antibiotic, designated kanamycin B. The two antibiotics are best separated in Peterson's *n*-butanol–water–2% *p*-toluenesulfonic acid system[5] on Schleicher and Schuell 589 Blue Ribbon or Whatman No. 1 papers. In this system with S&S 589 Blue Paper, kanamycin has an R_F of about 0.35 and kanamycin B has an R_F of about 0.6. The presence of impurities or contaminating salts interferes markedly with the paper chromatography of the kanamycins in this system.

The infrared spectra of kanamycin and kanamycin B are similar. Each is typical of a polyhydroxy, polyamino compound. No carbonyl or carbon–carbon double bond adsorption is evident.

Kanamycin is remarkably resistant to acid and alkaline hydrolysis. Treatment of kanamycin with methanolic hydrogen chloride under conditions which hydrolyze neomycin B and C to neomycin A (neamine)[6–8] yielded unchanged starting material. Refluxing kanamycin base in 6 *N* HCl

(1) T. Takeuchi, T. Hikiji, K. Nitta, S. Yamazaki, S. Abe, H. Takayama and H. Umezawa, *J. Antibiotics*, **A10**, 107 (1957).

(2) (a) H. Umezawa, M. Ueda, K. Maeda, K. Yagishita, S. Kondo, Y. Okami, R. Utahara, Y. Osato, K. Nitta and T. Takeuchi, *ibid.*, A10, 181 (1957); (b) K. Maeda, M. Ueda, K. Yagishita, S. Kawaji, S. Kondo, M. Murase, T. Takeuchi, Y. Okami and H. Umezawa, *ibid.*, A10, 228 (1957).

(3) A product of the Rohm and Haas Co.

(4) The X-ray determination was run at the Massachusetts Institute of Technology, through the courtesy of Dr. David P. Shoemaker.

(5) D. H. Peterson and L. M. Reinecke, THIS JOURNAL, **72**, 3598 (1950).

(6) J. D. Dutcher, N. Hosansky, M. N. Donin and O. Wintersteiner, *ibid.*, **73**, 1384 (1951).

(7) B. E. Leach and C. M. Teeters, *ibid.*, **73**, 2794 (1951).

(8) R. L. Peck, C. E. Hoffhine, Jr., P. Gale and K. Folkers, *ibid.*, **75**, 1018 (1953).