their associates for supplying pilot plant quantities of culture filtrates and crude concentrates; to Dr. M. W. Fisher for assays *vs. Mycobacterium tuberculosis* H37Rv; to Dr. John M. Vandenbelt and his associates for ultraviolet and infrared determinations; and to Mr. C. E. Childs and associates for microanalyses.

DETROIT 32, MICHIGAN

[CONTRIBUTION FROM THE RESEARCH DIVISION, PARKE, DAVIS & COMPANY]

Amicetin, Bamicetin and Plicacetin. Chemical Studies

BY THEODORE H. HASKELL

RECEIVED AUGUST 26, 1957

From acid hydrolysates of amicetin (I), cytimidine (II) and the N-dimethylaminoglycoside, amicetamine (III), have been isolated. Acid degradation of bamicetin yields cytimidine and the monomethylaminoglycoside, bamicetamine. Plicacetin has been synthesized from cytosamine (IV) and *p*-nitrobenzoyl chloride, followed by reduction of the nitro group. Other microbiologically active derivatives have been prepared and the structural requirement for activity deduced.

The isolation and characterization of amicetin and two novel basic antibiotics, bamicetin and plicacetin, was described in a previous publication.[1] The three crystalline substances were elaborated by an actinomycete designated as *Streptomyces plicatus*. This paper describes some studies on the structural interrelationships of the three antibiotics, the synthesis of plicacetin from the alkaline degradative fragment, cytosamine, and the preparation of some microbiologically active derivatives.

The proposed structural formulas for amicetin and plicacetin as well as the partial formula for bamicetin were presented in the previous publication.[1] These were deduced by comparing the acidic and basic degradative fragments of plicacetin and bamicetin with those of amicetin (I) as reported by Flynn and co-workers.[2] Since alkaline hydrolysis of plicacetin yielded only cytosamine (IV) and *p*-aminobenzoic acid, its structural relationship to amicetin was fairly well established. This has now been confirmed by synthesis. Acid hydrolysis of bamicetin resulted in the liberation of cytimidine (II) indicating that its difference from the amicetin molecule resides in its unknown basic glycosidic moiety.

The aminoglycosides from amicetin and bamicetin have been isolated in pure form, using the hydrolytic conditions previously described for the formation of cytimidine. The glycosidic fragment from amicetin, referred to as amicetamine (III),[3] was isolated as both the amorphous free base and as the crystalline hydrochloride. The glycosidic fraction from bamicetin, designated as bamicetamine, was isolated from an acid hydrolysate as the amorphous free base and has been assigned an empirical formula $C_{13}H_{25}NO_6$ on the basis of microanalysis and potentiometric titration data. Both amine functions in these glycosides had apparent dissociation constants of about 7.0. Amicetamine free base was found to contain two acetylable hydroxyl groups, approximately two N-methyl and C-methyl groups and no methoxyl. Both glycosidic components from amicetin and bamicetin gave positive iodoform tests and possessed a potential carbonyl group as determined by hydroxylamine titration.[4] However, they failed to reduce Fehling or Benedict solution and did not give any of the carbohydrate color tests based on furfural formation. Furthermore, they failed to absorb hydrogen with Adams catalyst in glacial acetic acid. Both infrared spectra showed absorption bands at 2.94 and 3.0 μ (hydroxyl), strong ether bands in the 8.7–9.6 μ region and no absorption in the carbonyl region.

Periodate oxidation studies on the amicetin and bamicetin free base glycosides showed an uptake of approximately three moles of oxidant in 24 hr. in each case. From the oxidation mixture of amicetamine, dimethylamine, formic acid and glyoxal were isolated, the first two in approximately equivalent proportions. Dimethylamine was characterized as its crystalline *p*-hydroxyazobenzene *p'*-sulfonate salt, formic acid as its barium salt and glyoxal as its phenylosazone derivative. From the bamicetamine oxidation mixture, monomethylamine and formic acid were isolated. It thus appears that one major difference between the structure of amicetin and bamicetin resides in the amine function of their glycosidic moieties.

The isolation and characterization of low molecular weight volatile amines as their *p*-hydroxyazobenzene *p'*-sulfonate salts appears to be a very convenient and reliable method. These bases on liberation by periodate oxidative methods can be adsorbed on Dowex-50 columns, eluted with mineral acids, and, after making alkaline, steam distilled from the Kjeldahl apparatus into aqueous solutions of the dye acid. The salts formed can be readily crystallized from water, possess sharp characteristic melting or decomposition points and can be quantitatively analyzed by spectrophotometric procedures. Further confirmation can be obtained by comparing their infrared spectra with authentic samples. Table I lists some comparative data obtained with several low molecular weight volatile amines.

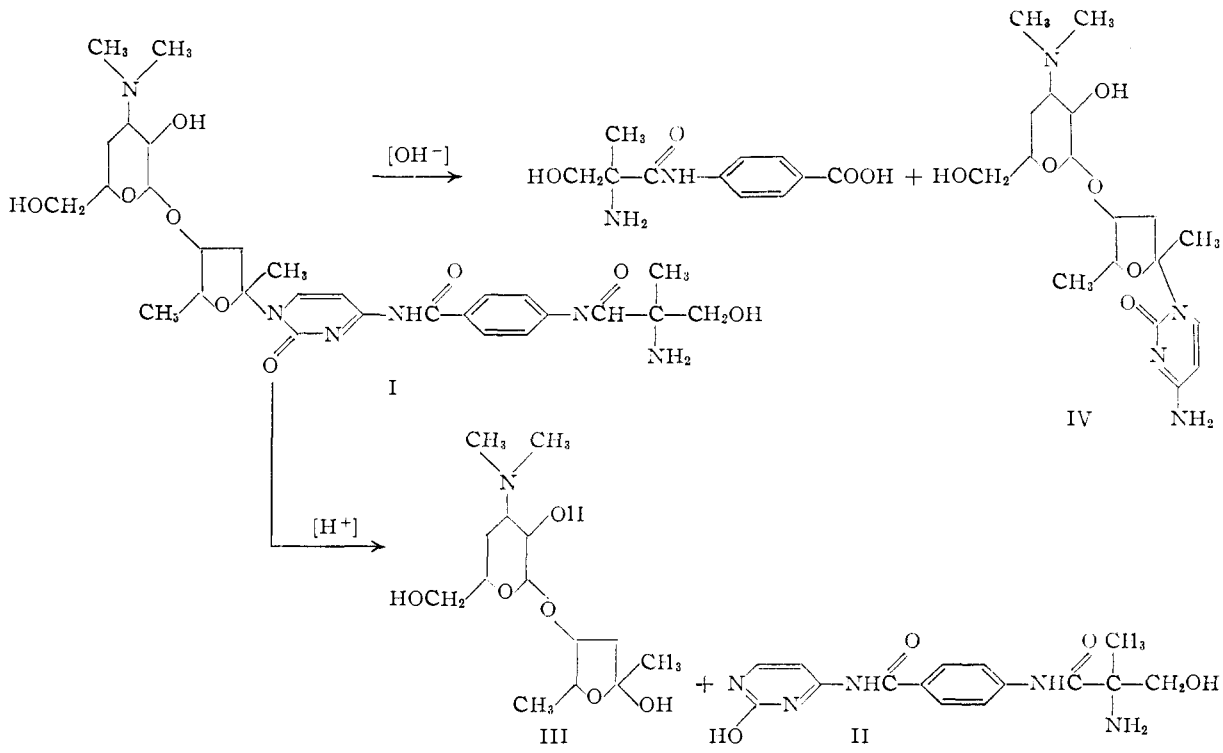
By establishing the presence of a tertiary amine function in amicetamine, the possibility of forming synthetic amicetin-like derivatives by N^6-acylation of cytosamine became apparent. The first such

(1) T. H. Haskell, A. Ryder, R. P. Frohardt, S. A. Fusari, Z. L. Jakubowski and Q. R. Bartz, THIS JOURNAL, **80**, 743 (1958).

(2) E. H. Flynn, J. W. Hinman, E. L. Caron and D. O. Woolf, Jr., *ibid.*, **75**, 5867 (1953).

(3) C. L. Stevens, R. J. Gasser, T. Mukherjee and T. H. Haskell, *ibid.*, **78**, 6212 (1956).

(4) A. R. Trim and R. Hill, *Biochem. J.*, **50**, 314 (1952).



derivative attempted was the antibiotic plicacetin. This was accomplished successfully by condensation of *p*-nitrobenzoyl chloride with finely suspended cytosamine in refluxing chloroform. The crystalline N^6-*p*-nitrobenzoylcytosamine, obtained in 65% yield, was reduced with palladium-on-charcoal catalyst to give the corresponding *p*-amino compound. This was found to be identical in all respects to plicacetin. Other aroyl derivatives prepared using this heterogeneous reaction system include the N^6-benzoyl-, *m*-nitrobenzoyl- and 3,5-dinitrobenzoylcytosamine. The yields, however, were substantially lower than obtained with the *para*-nitro isomer.

TABLE I

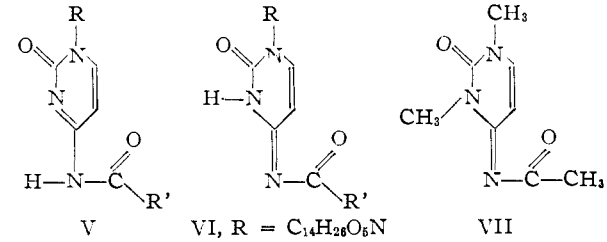
Base	M.p. °C.	*a* (calcd.)[a]	*a* (found)
NH_3	275-280	76.1	75.6
CH_3NH_2	239-241	72.7	72.5
$(CH_3)_2NH$	217-218	69.5	70.0
C_2H_5-NH_2	233-235	69.5	70.6

[a] *a* values were calculated on the basis of an absorbancy of 80.8 for the free dye acid at λ_{max} 355 mμ in 0.1 *M* phosphate buffer at *p*H 7.0. All of the above salts showed maximum absorption at this wave length.

N-Acylation of the pyrimidine derivative could also be accomplished with aliphatic acid anhydrides. In the few attempts described, however, ester formation also occurred as evidenced by the appearance of 5.7 and 5.78 μ bands in the infrared spectra. Crystalline derivatives corresponding to diacetyl, dipropionyl and triacetyl cytosamine were isolated. Although the disubstituted derivatives are probably non-homogeneous as suggested from analytical and melting point data, it appears that ester formation occurs on the hydroxyl group adjacent to the tertiary amine. This was suggested by the lowering of the apparent dissociation constant of the amine function by more than two pK_a' units on esterification.[5]

To demonstrate that N^6-acylation had occurred in each case, potentiometric titration data and ultraviolet absorption spectra were studied. The presence of a weakly acidic group[2] with an apparent dissociation constant of 8.7 to 12 and the presence of two absorption maxima in acidic media at λ values of 240–265 and 307–312 mμ were indicative of amide formation. The starting material, cytosamine, has no acidic hydrogen (pK_a' values of 4.1 and 7.2) and absorbs in aqueous acid at λ values of 212 and 277 mμ.

All of the N^6-acylated cytosamine derivatives prepared to date have been found to be microbiologically active exhibiting an antibacterial spectrum similar to amicetin. Some properties of these derivatives are listed in Table II. Since cytosamine is devoid of bioactivity, it appears evident that N^6-acylation is necessary for antibacterial action. Such acylated compounds can exist as tautomeric structures V and VI.



V VI, R = $C_{14}H_{26}O_5N$ VII

Kenner and co-workers[6] have studied the acylated derivatives of 3-methylcytosine and have conclusively demonstrated that the position of the

(5) E. H. Flynn, M. V. Sigal, P. F. Wiley and K. Gerzon, THIS JOURNAL, **76**, 3121 (1954).

(6) G. W. Kenner, C. B. Reese and A. R. Todd, *J. Chem. Soc.*, 855 (1955).

TABLE II

N^6-SUBSTITUTED CYTOSAMINE DERIVATIVES

Acyl substituent	M.p. °C.	Bio-assay[a]	pKa'[b]	λ_{max} mμ[c]
p-Nitrobenzoyl	147–149	392	6.9, 10.3	265, 310
m-Nitrobenzoyl	128–130	225	7.0, 11.0	256, 311
3,5-Dinitrobenzoyl	140–143	270	6.8, 8.7	247, 310
Benzoyl	130–135	197	7.0, 10.5	258, 312
Acetyl (di)	149–153	284	4.6, 12.4	240, 307
Acetyl (tri)	216–217	230	4.2, 12.3	239, 307
Propionyl (di)	124–128	59	4.6, 12.4	238, 307

[a] Microbiological potencies as measured against *E. coli* PD 04863 are expressed as micrograms per milligram of crystalline amicetin base. [b] Determined in aqueous ethanol solutions. [c] Determined in 0.1 *N* hydrochloric acid.

acyl residue as well as that of the mobile hydrogen atom corresponds to structure V. By comparison of ultraviolet absorption data of various N^6-acetylated cytosine derivatives these authors suggested structure V to be the predominant tautomer. Structure VI (R = CH_3), which is the more acidic form, was shown to react with diazomethane in alcoholic solution with formation of N^6-acetyl-1,3-dimethylcytosine (VII). The compound N^6-*p*-nitrobenzoylcytosamine has been subjected to diazomethane treatment under conditions identical to those described by Kenner. From the reaction mixture there was isolated an amorphous orange powder showing no acidic hydrogen function by titration and which was antibacterially inert. Although the exact composition of this reaction product is unknown, it appears plausible to assume that the 1-methyl derivative was formed and that the acidic hydrogen function is indeed an absolute requirement for biological activity with this type of antibiotic.

Experimental

Alkaline Hydrolysis of Plicacetin.—Plicacetin (0.10 g.) was wetted with two drops of methanol and diluted to 2.5 ml. with 0.1 *N* sodium hydroxide. The white crystalline precipitate which formed after standing at room temperature for 48 hr. was removed by filtration and washed with a few drops of ice-water. Upon addition of 1.2 ml. of 6 *N* sodium hydroxide to the filtrate and cooling, a second crop of crystals was obtained. After drying *in vacuo*, the combined crops (0.059 g.) were recrystallized from absolute ethanol–ethyl acetate solution. The product was identified as cytosamine by melting point, ultraviolet absorption and infrared absorption spectra.

The alkaline filtrate after removal of cytosamine was adjusted to 5 ml. with water and heated to 100° for 1 hr. On cooling the solution was adjusted to *p*H 3.1 with sulfuric acid and extracted four times with 3-ml. portions of ethyl acetate. The combined ethyl acetate extracts upon evaporation and drying *in vacuo* yielded 0.023 g. of crystalline residue. The product after recrystallization from aqueous ethanol melted at 185–186° and showed no depression on admixture with authentic *p*-aminobenzoic acid.

The aqueous layer after ethyl acetate extraction failed to give a ninhydrin test. Amicetin, when subjected to this treatment gave cytosamine, *p*-aminobenzoic acid and α-methylserine.

Acid Hydrolysis of Bamicetin.—The procedure of Flynn and co-workers[2] for the isolation of cytimidine from amicetin was employed. From 0.34 g. of bamicetin, 0.12 g. (67%) of crystalline cytimidine-free base melting at 265° dec. was obtained. Its ultraviolet and infrared absorption spectra were identical with those of cytimidine obtained from amicetin.

Anal. Calcd. for $C_{15}H_{17}N_5O_4$: C, 54.37; H, 5.17; N, 21.14. Found: C, 54.25; H, 5.44; N, 21.21.

Amicetamine from Amicetin.—Amicetin (6 g.) was dissolved in 140 ml. of 6 *N* hydrochloric acid and warmed to 70° with stirring for five minutes. The solution was cooled and the crystalline cytimidine hydrochloride removed by filtration. Excess acid was removed from the filtrate by azeotropic distillation with 1-butanol *in vacuo*. Care was taken to maintain the temperature below 30° to prevent charring. When the flask contents reached a volume of about 20 ml., additional cytimidine hydrochloride was removed by filtration and the filtrate diluted with 100 ml. of water. The solution was further deacidified by passage over a column containing 23 ml. of Amberlite IR-45 (OH form). The effluent and washings then were passed through a column containing 60 ml. of Dowex-50-X8 (50–100 mesh) and the column washed with 200 ml. of water. Elution was accomplished with 1.5 *N* hydrochloric acid, 25-ml. fractions being collected with an automatic fraction collector. All fractions were checked for ultraviolet absorbancy in the 260–280 mμ region. Fractions 1 to 9 showed no absorbancy maximum in this region and were combined. Fractions 10 through 19 absorbed at a maxima of 275 mμ and contained a few mg. of cytosine.

The combined fraction 1 through 9 was concentrated *in vacuo*, reconstituted in water and passed over a column containing 18 ml. of Dowex 50 X-2 (50–100 mesh). The column was washed with 100 ml. of water and eluted with 0.15 *N* ammonium hydroxide. The alkaline eluate (*ca.* 200 ml.) was concentrated *in vacuo* to remove excess ammonia and lyophilized. White amorphous amicetamine-free base (1.6 g.) was obtained; $[\alpha]^{26}D$ +124° (*c* 1% in 0.1 *N* hydrochloric acid); pK'_a = 7.0 (H_2O); mol. wt. from titration, 306.

Anal. Calcd. for $C_{14}H_{27}NO_6$ (mol. wt. 305.4): C, 55.06; H, 8.91; N, 4.59; hydroxyl value (2-OH), 10.07; N-methyl (2) 9.8. Found: C, 54.58; H, 9.09; N, 4.76; hydroxyl value, 10.55; N-methyl, 7.8; C-methyl, 7.7.

Amicetamine Hydrochloride.—Amicetamine-free base (1.33 g.) was dissolved in 30 ml. of water, the *p*H adjusted to 5.6 by the addition of hydrochloric acid and the solution lyophilized. The residue, upon addition of 10 ml. of absolute ethanol and gentle warming, solidified to a crystalline mass. The crystals were removed by filtration, washed with cold absolute ethanol followed by acetone and dried *in vacuo*. The product weighed 0.73 g. and melted at 168–170°. An additional 0.40 g. was obtained from the concentrated mother liquors upon addition of acetone.

Anal. Calcd. for $C_{14}H_{27}NO_6 \cdot HCl$: C, 49.19; H, 8.26; N, 4.10; Cl, 10.37. Found: C, 49.14; H, 8.40; N, 4.12; Cl (ionic), 10.45.

Bamicetamine from Bamicetin.—The glycosidic component was isolated from bamicetin by the same procedure as described for amicetamine-free base. The antibiotic (3.0 g.) afforded 0.78 g. of white free base lyophilizate. On treatment of this product with absolute ethanol the anhydrous form precipitated as a white powder, which melted with decomposition at 229–231°; $[\alpha]^{26}D$ +155° (*c* 1% in 0.1 *N* hydrochloric acid).

Anal. Calcd. for $C_{13}H_{25}NO_6$: C, 53.59; H, 8.65; N, 4.81. Found: C, 53.76; H, 8.52; N, 5.05; ash, 0.76.

Periodate Oxidation of Amicetamine.—Sodium metaperiodate in buffered and unbuffered solutions was used and the uptake of oxidizing agent was determined using the arsenite procedure.[7] In both buffered and unbuffered solutions amicetamine consumed 2.6 moles of oxidant in 30 minutes and 2.9 moles per mole in 24 hr.

A. Isolation of Volatile Base.—Amicetamine (0.32 g.) was added to a solution of 0.65 g. (3 equiv.) of sodium metaperiodate in 10 ml. of water. After standing at room temperature for 1 hr., the mixture was passed through a column containing 10 ml. of Dowex 50 X 12 (100–200 mesh) and rinsed with water. The effluent and washings were saved for the isolation of the volatile acid. The column was eluted with about 60 ml. of 2 *N* hydrochloric acid and the eluate concentrated to dryness *in vacuo*. The residue was reconstituted in a small volume of water, transferred to a micro-Kjeldahl-flask and after making alkaline subjected to steam distillation. The distillate was collected in a solution containing 0.28 g. of *p*-hydroxyazobenzene-*p'*-sulfonic acid in 10 cc. of water. The mixture was concentrated to dryness *in vacuo* and the residue crystallized from water. The product weighed 0.2 g. and melted at 217–218°. Admixture with an authentic sample of dimethylammonium *p*-hydroxy-

(7) R. Adams, *et al.*, "Organic Reactions II," 1st Ed., John Wiley and Sons, Inc., New York, N. Y., 1944, Chapter 8.

azobenzene *p'*-sulfonate showed no melting point depression. The salt exhibited an absorbancy of 68.8 at λ_{max} 355 mμ at *p*H 7.0 and had an infrared absorption spectrum identical to that of an authentic sample.

B. Isolation of Volatile Acid.—The combined effluent and washings from the Dowex 50 column was adjusted to *p*H 8.5 with barium hydroxide and the precipitate removed by filtration. The filtrate was concentrated *in vacuo* to a volume of about 10 ml. and filtered. The *p*H of the filtrate was lowered to 2.0 with 1 *N* sulfuric acid and the barium sulfate removed by centrifugation. The supernatant was then distilled to a volume of approximately 3 ml. Water (10 ml.) was added and the distillation continued to almost dryness. The distillate on titration with standard barium hydroxide showed the presence of 0.7 mmole of volatile acid. The titrated solution on lyophilization gave 0.077 g. of white powder which was recrystallized ffom aqueous ethanol.

Anal. Calcd. for $(CHO_2)_2Ba$: Ba, 60.35. Found: Ba, 59.92.

C. Isolation of Glyoxal.—To 1.0 g. of sodium metaperiodate dissolved in 6 ml. of water was added 0.50 g. of amicetamine-free base. After standing at room temperature for 1 hr., 50 ml. of absolute ethanol was added and the salt removed by filtration. To the filtrate was added 0.56 g. of phenylhydrazine hydrochloride and 0.5 g. of sodium acetate and the mixture refluxed for 1 hr. The mixture was concentrated to dryness *in vacuo*, suspended in water, extracted with benzene and the benzene layer dried over anhydrous sodium sulfate. The filtered benzene solution was diluted with a half volume of *n*-heptane and chromatographed over a column containing 200 g. of acid-washed alumina. The main yellow band obtained was rechromatographed over another 200 g. of alumina column. From this yellow band there was obtained 0.158 g. of crystalline material which, on repeated recrystallization from ethyl acetate–heptane solution gave a product which melted at 170–173°. The phenylosazone of glyoxal melts at 174–176°.

Anal. Calcd. for $C_{14}H_{14}N_4$: C, 70.56; H, 5.92; N, 23.51. Found: C, 70.53; H, 5.71; N, 23.40.

Periodate Oxidation of Bamicetamine.—Oxidative conditions similar to those described for amicetamine were used. Quantitative studies showed an uptake of 2.6 moles of oxidant per mole of glycoside after 4 hr. and 3.2 moles after 24 hr.

A. Isolation of Volatile Amine.—The volatile base was isolated by the same procedure as previously described. The dye salt obtained melted at 239–241° and showed no depression on admixture with an authentic sample of monomethylammonium *p*-hydroxyazobenzene *p'*-sulfonate. The salt had an absorbancy of 72.4 at λ 355 mμ at *p*H 7.0. Comparison of infrared curves further identified the base as monomethylamine.

B. Isolation of Volatile Acid.—Barium formate (0.9 mmole) was isolated from the oxidized mixture of bamicetamine by the previously outlined procedure.

Anal. Calcd. for $Ba(CHO_2)$: Ba, 60.35. Found: Ba, 60.23.

Acylation of Cytosamine

Anhydrous Cytosamine.—Cytosamine, reported by Flynn and co-workers[2] as melting at 160–165°, is the hydrated form. Recrystallization from hot absolute ethanol gave the anhydrous form as white needles melting at 252–255° with decomposition. Ultraviolet absorption showed maximum *a* values of 28.0 and 35.0 at λ 212 and 277 mμ, respectively, in 0.1 *N* hydrochloric acid solution; $[\alpha]^{25}D$ +107° (*c* 1% in 0.1 *N* hydrochloric acid).

Anal. Calcd. for $C_{18}H_{30}N_4O_6$: C, 54.26; H, 7.59; N, 14.06. Found: C, 54.21; H, 7.76; N, 14.26.

N^6-*p*-Nitrobenzoylcytosamine.—Finely divided anhydrous cytosamine (3 g.) was suspended in 150 ml. of chloroform and the mixture refluxed with stirring. To this was added a solution of 1.5 g. of *p*-nitrobenzoyl chloride in 50 ml. of chloroform and the resulting suspension refluxed for 16 hr. The precipitate was removed by filtration, washed with chloroform and dried *in vacuo*. The dried product (4.3 g.) was suspended in 50 ml. of water and the *p*H raised to 8.3 by the addition of alkali with vigorous stirring. The product, after filtration and rinsing with water, was recrystallized from 40 ml. of absolute ethanol. Light yellow platelets (2.5 g.), melting at 147–149°, were obtained. An additional 0.20 g. of crystalline material was obtained from the mother liquors (65% yield). The product showed maximum *a* values of 40.8 and 30.7 at λ values of 265 and 310 mμ, respectively, in 0.1 *N* hydrochloric acid.

Anal. Calcd. for $C_{25}H_{33}O_9N_5$: C, 54.84; H, 6.07; N, 12.79. Found: C, 54.53; H, 6.22; N, 12.69.

N^6-*p*-Aminobenzoylcytosamine (Plicacetin).—N^6-*p*-Nitrobenzoylcytosamine (2.5 g.) was dissolved in 200 ml. of a 70% ethanol–water mixture which contained 8 mmoles of hydrochloric acid. Approximately 0.3 g. of a 5% palladium-on-charcoal catalyst was added and the contents reduced in a Parr hydrogenator at 55 lb. for 1.5 hr. The catalyst was removed by filtration, 2 ml. of 2 *N* alkali added to the filtrate and the contents concentrated *in vacuo* to remove ethanol. The suspension was reconstituted in 200 ml. of water, the *p*H raised to 8.2 with alkali and dried from the frozen state. The product (2.6 g.) was stirred with 10 ml. of ice-water, filtered and dried *in vacuo*. The light pink amorphous product (1.7 g.) was recrystallized from methanol–ethyl acetate affording 1.4 g. of needles melting at 158–162°. Ultraviolet absorption spectra, bioassay, infrared spectra and titration data indicated that the *p*-aminobenzoyl derivative was identical with plicacetin. It was also readily convertible into the other two crystalline forms.[1]

Anal. Calcd. for $C_{25}H_{35}N_5O_7$: C, 58.01; H, 6.82; N, 13.53. Found: C, 57.80; H, 6.82; N, 13.73.

N_6-Aroyl Cytosamine Derivatives.—Other aroyl substituted cytosamine derivatives were prepared from the corresponding acid chlorides using the same reaction conditions described for the N^6-*p*-nitrobenzoyl derivative.

N_6-Benzoylcytosamine.—This derivative was obtained as colorless needles after recrystallization from aqueous ethanol. The product, obtained in 10% yield, melted at 130–135° and had maximum *a* values of 32.9 and 35.6 at λ 258 and 312 mμ, respectively, in 0.1 *N* hydrochloric acid.

Anal. Calcd. for $C_{25}H_{34}N_4O_7$: N, 11.15. Found: N, 11.20.

N_6-*m*-Nitrobenzoylcytosamine.—The product was obtained as light yellow needles which, after several recrystallizations from aqueous ethanol, melted at 128–130°. The purified derivative, obtained in 8% yield, had maximum *a* values of 41.0, 43.7 and 24.7 at λ 218, 256 and 311 mμ, respectively, in 0.1 *N* hydrochloric acid.

Anal. Calcd. for $C_{25}H_{33}O_9N_5$: N, 12.79. Found: N, 12.59.

N_6-3,5-Dinitrobenzoylcytosamine.—This derivative was obtained as yellow needles after recrystallizing from 95% ethanol. The purified product, obtained in 4% yield, melted at 140–143° and had maximum *a* values of 52.7, 46.2 and 19.4 at λ 215, 247 and 310 mμ, respectively, in 0.1 *N* hydrochloric acid.

Anal. Calcd. for $C_{25}H_{32}O_{11}N_6$: N, 14.18. Found: N, 14.58.

Action of Acid Anhydrides on Cytosamine

N_6,O-Diacetylcytosamine.—To 500 mg. of cytosamine was added 1.5 ml. of acetic anhydride and the mixture warmed slightly to effect complete solution. After standing at room temperature overnight the excess anhydride was removed *in vacuo*, the residue dissolved in water and the solution lyophilized. The white solid obtained was suspended in 6 ml. of water and the *p*H raised to 7.5 with alkali. The white granular precipitate on filtration, washing with water and drying *in vacuo*, weighed 0.49 g. and melted between 142–147°. Recrystallization from aqueous ethanol raised the melting point to 149–153°. The homogeneity of this preparation is somewhat doubtful since variable melting points were obtained on further recrystallization from aqueous ethanol. The colorless needles obtained gave maximum *a* values of 25.9, 17.6 and 27.5 at λ 212, 240 and 307 mμ in 0.1 *N* hydrochloric acid. Microanalytical values given below are averages obtained from 5 separate analyses.

Anal. Calcd. for $C_{22}H_{34}N_4O_8$: C, 54.76; H, 7.10; N, 11.61. Found: C, 54.23; H, 7.31; N, 11.25.

N^6-O,O-Triacetylcytosamine.—To 1.0 g. of cytosamine was added 5 ml. of acetic anhydride and the clear solution heated for 6 hr. on a steam-bath. The excess acid and anhydride was removed *in vacuo*, the residue reconstituted in water and dried from the frozen state. The white product obtained was suspended in 10 ml. of water and the *p*H adjusted to 7.0 by the addition of alkali with vigorous stirring.

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 *p*H 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 *p*H 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.

Acknowledgments.—The author wishes to express his appreciation to Mrs. Alyce Hammond and Mr. Charles George for valuable technical assistance; to Dr. John M. Vandenbelt and his associates for ultraviolet and infrared determinations; to Mr. C. E. Childs and associates for microanalyses; and to Dr. John Ehrlich and Dr. M. W. Fisher for microbiological assays.

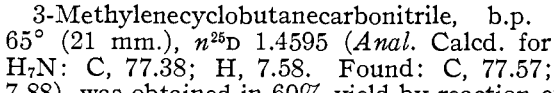
DETROIT 32, MICHIGAN

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).