Ammonoacetals. I. A New Class of Potential Antineoplastic Compounds[1]

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Ammonoacetals may be considered as alkylating agents in chemical systems and their use is now considered in biological systems for the chemotherapeutic treatment of tumors. A new class of ammonoacetals, the bipiperidinoimidazolidines, has been prepared and its properties described.

Ammonoacetals, >NCR$_2$N<, have long been known,[3] and their preparation and properties described. With the exception of Wagner and his co-workers,[4] there has been little systematic investigation of these compounds as a class or recognition that such a grouping may have discrete properties. A study of the literature, however, does show that they can act as alkylating agents on active carbon atoms, amines, and phosphines[4,5] either by the transfer of the >NCHR– (R = H, alkyl, or aryl) grouping or by the transfer of a one-carbon fragment. Hunt and Wagner[4c] have shown that ammonoacetals, Schiff bases, and methylolamino compounds react in aqueous solution through a common intermediate which is readily formed by protons present in low concentration and even in buffered solutions (pH 7). Such compounds might therefore be active under physiological conditions.

Compounds in this category are known to occur biologically, Schiff bases in particular being of importance in a large number of enzymatic reactions.[6] Also, the adduct between formaldehyde and tetrahydrofolic acid, N^5,N^{10}-methylene tetrahydrofolate ("active formaldehyde"),[7] is an ammonoacetal, acting *in vivo* by the transfer of a one-carbon fragment and whose acid sensitivity is explainable in terms of this ammonoacetal structure. In addition, it is also possible that the tumorcidal action of the antifolic acid drugs such as aminopterin[8] may stem in part from the possible alkylating action of a methylol derivative.

It would therefore seem that these compounds might be of use in the chemotherapeutic treatment of neoplasms. Hendry, *et al.*,[9] have found, in fact, that 2,4,6-trimethylolmelamine showed tumor inhibitory action against Walker 256 carcinoma in rats, and more recently Billman and Meisenheimer[10] have prepared and have begun to investigate the tumorcidal action of several hexahydropyrimidines. These are the few reports relevant to the use of this general class of compound.

The present work was undertaken to obtain more information about the ammonoacetal system and to prepare several representative ammonoacetals for chemical and biological evaluation. In the course of this study a new class of heterocycle, the bipiperidino(1,2-*c*:2′,1′-*e*)imidazolidines (III), was prepared. This was achieved by the reaction between 2,2′-bipiperidine (I) and an aldehyde, followed by cyclization of the intermediate methylol adduct II. In this manner bipiperidino(1,2-*c*:2′,1′-*e*)imidazolidine (IIIa) and the 2-methyl (IIIb) and 2-phenyl (IIIc) derivatives were prepared. Attempts to synthesize 2,2′-disubstituted bipiperidinoimidazolidines by the reaction between amine I and ketones such as acetophenone and 2-butanone were singularly unsuccessful. However, there are few recorded instances of the reaction between 1,2-diamines and ketones yielding imidazolidines.[11] This is presumably due to the lower reactivity of ketones, and to the increased steric strain, as observed with molecular models, which is inherent in such disubstituted bipiperidinoimidazolidines.

The three imidazolidines (IIIa–c) were colorless oils, water-souble with the exception of the phenyl compound and comparatively stable when heated as shown by their behavior on distillation. Addition of ethereal hydrogen chloride to solutions of these bases gave white, flocculent precipitates of the hydrochlorides, but these were so deliquescent that they were not further investigated. The formaldehyde compound IIIa gave a stable crystalline picrate, but the benzaldehyde derivative gave only a gum which could not be induced to crystallize. The preparation of these compounds in aqueous solution, their comparative stability to heat, and the formation of salts without immediate cleavage of the N–C–N bonds show that they are more stable than the majority of monocyclic imidazolidines, and considerably more stable than linear ammonoacetals.[4c,11,12] These compounds also differ from linear ammonoacetals containing the piperidine moiety in that they do not undergo cleavage when treated with alkaline hydrogen peroxide.[13]

Bergmann, *et al.*,[11] state that the infrared spectrum of the grouping N–C–N is characterized by three peaks occurring between 1089 and 1170 cm.$^{-1}$. The spectra of these imidazolidines III in KBr disks were found to have similar absorption bands: IIIa absorbing at 1109,

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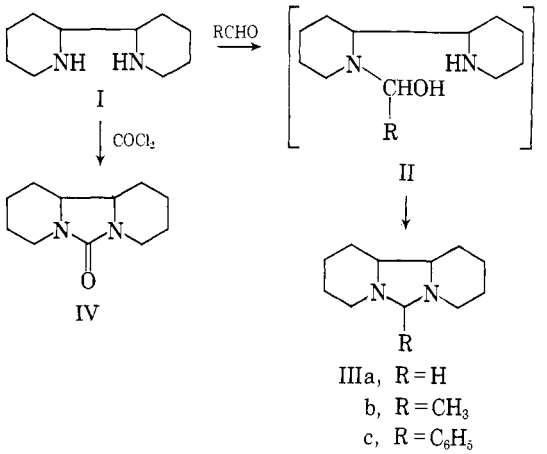
1135, 1151, and 1170 cm.$^{-1}$ (shoulder); IIIb at 1094, 1132, and 1150 cm.$^{-1}$; and IIIc at 1108, 1135, 1150, and 1170 cm.$^{-1}$. These maxima presumably correspond to the C–N frequency.

Reaction of the amine I with phosgene gave the imidazolidone, bipiperidino(1,2-*c*:2′,1′-*e*)imidazolidin-2-one (IV). This was a very pale yellow oil at room temperature, forming a white solid when kept below 0°. It was rather unstable, darkening on standing if not refrigerated, and was comparatively soluble in water giving a slightly acidic solution. Assignment has been made only for the carbonyl peak, 1715 cm.$^{-1}$, in the complex infrared spectrum. The normal value for fully substituted ureas, including 2-imidazolidone,[14] is about 1660 cm.$^{-1}$, but Randall, *et al.*,[15] found that in 1-methyl and 3-methylhydantoin the 2-carbonyl absorption occurs at 1712 and 1706 cm.$^{-1}$, respectively. These high values, and also the value for the imidazolidone IV, are undoubtedly due to the considerable strain present in these two ring systems.



Biological Results.—In addition to the biological evaluation of the bipiperidino(1,2-*c*:2′,1′-*e*)imidazolidines (III), other ammonoacetals were also screened against a subcutaneously transplanted murine ependymoblastoma.[16] No tumor regression was observable with the above imidazolidines, bipiperidinomethane, and the hydroxymethylol derivative of phthalimide. Slight, if any, effect was obtained with phthalimidopiperidinomethane and inconclusive but slight effect did occur with succinimidopiperidinomethane. Very large doses were required with the latter compound (300–400 γ/g.) and at this level an LD_{50} or greater effect was observed with these C_3H tumor-bearing mice.

Bone marrow depression studies were also performed,[17] and of the compounds examined only succinimidopiperidinomethane and bipiperidinomethane did have some slight effect. The latter compound was the more pronounced of the two.

Experimental

All analyses, including molecular weight determinations performed in benzene solution, and the measurement of infrared spectra were performed by Schwarzkopf Microanalytical Laboratories, New York, N. Y. Vacuum distillation of the imidazolidines III was complicated by foaming and in all cases a larger distillation pot than normal was used.

2,2′-Bipiperidine (I).—Commercially available 2,2′-bipyridyl (10 g.) in glacial acetic acid (35 ml.) and concentrated hydrochloric acid (25 ml.) was shaken with platinum oxide (500 mg.) under hydrogen for 24 hr. The filtered solution was strongly basified with 60% sodium hydroxide solution and extracted with five 100-ml. portions of ether. Distillation of the dried ethereal solution gave 2,2′-bipiperidine (8.6 g., 80%), b.p. 131–132° (20 mm.).[18]

Preparation of the Imidazolidines (IIIa–c).—The amine I (3.4 g., 0.02 mole) was dissolved in water (150 ml.) and a solution or suspension of the appropriate aldehyde (0.02 mole) in water (30 ml.) was added with stirring. Stirring was continued for 30 min. at room temperature and then for a further 45–60 min. on a steam bath. The cooled solution was extracted with three 150-ml. portions of ether, the combined ethereal extracts were dried over anhydrous sodium sulfate, and the imidazolidine was distilled after removal of the solvent.

Bipiperidino(1,2-*c*:2′,1′-*e*)imidazolidine (IIIa) had b.p. 246–248° dec. and 102–104° (12 mm.), yield 70% after purification.

Anal. Calcd. for $C_{11}H_{20}N_2$: C, 73.3; H, 11.2; N, 15.5; mol. wt., 180.2. Found: C, 73.4; H, 11.3; N, 15.5; mol. wt., 188.

2-Methylbipiperidino(1,2-*c*:2′,1′-*e*)imidazolidine (IIIb) had b.p. 128–130° (16 mm.), yield 55% after purification.

Anal. Calcd. for $C_{12}H_{22}N_2$: C, 74.2; H, 11.4; mol. wt., 194.2. Found: C, 74.1; H, 11.6; mol. wt., 194.

2-Phenylbipiperidino(1,2-*c*:2′,1′-*e*)imidazolidine (IIIc) had b.p. 111–112° (0.25 mm.), yield 65% after purification.

Anal. Calcd. for $C_{17}H_{24}N_2$: C, 79.6; H, 9.4; N, 10.9; mol. wt., 256.2. Found: C, 79.7; H, 9.4; N, 10.9; mol. wt., 260.

This compound, like IIIa and IIIb was a mobile oil at room temperature and could not be induced to crystallize, even though benzylidenebispiperidine is a white crystalline solid, m.p. 80–81°.[19]

Treatment of the amine I with acetophenone as outlined above, returned only unchanged starting materials. The same results were obtained after repetition of the experiment in toluene using a Dean–Stark distilling apparatus for the removal of water and in benzene with calcium sulfate added as desiccant.

Imidazolidine Salts.—The dropwise addition of anhydrous ethereal hydrogen chloride to anhydrous ethereal solutions of the imidazolidines III gave flocculent, white precipitates of the respective hydrochlorides. These were so extremely deliquescent, however, that they were not examined further. Treatment of the imidazolidine IIIa with aqueous picric acid gave the **monohydrogen picrate**, which, after recrystallization from methanol, had m.p. 144–145°.

Anal. Calcd. for $C_{11}H_{20}N_2 \cdot C_6H_3N_3O_7$: C, 49.9; H, 5.7. Found: C, 50.1; H, 5.8.

Bipiperidino(1,2-*c*:2′,1′-*e*)imidazolidin-2-one (IV).—A solution of bipiperidine (4.0 g., 0.024 mole) and triethylamine (6.0 g., 0.06 mole) in toluene (40 ml.) was cooled in an ice bath, and a solution of phosgene (2.35 g., 0.024 mole) in toluene (25 ml.) also cooled to 0° was added with stirring over a period of 45 min. When addition was complete, a further portion of toluene (20 ml.) was added, and the suspension was heated slowly until it was boiling under gentle reflux, then cooled and extracted with two 50-ml. portions of water. Distillation of the dried toluene solution gave the imidazolidone IV; yield 2.8 g., 60%, b.p. 98–100° (0.25 mm.). An analytically pure sample of this compound retained a pale yellow coloration after three distillations.

Anal. Calcd. for $C_{11}H_{18}N_2O$: C, 68.0; H, 9.3; N, 14.4; mol. wt., 194.2. Found: C, 68.2; H, 9.4; N, 14.6; mol. wt., 199.

Biological Studies.—The method for determining tumor regression has been previously described[16] as well as the technique for measuring bone marrow depression.[17]

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