(20 ml) but omitting NaOH. Heating on the steam bath for 1 hr gave a clear solution which was cooled and diluted with dry ether (150 ml). The gummy precipitate crystallized on trituration with fresh ether giving 16.2 g, mp 141–145°. Recrystallization from ethanol gave a sample, mp 150–154°, which could not be completely purified: ν_{max}^{Nujol} 1132, 1302 (SO_2), 1083 (SO_2H), 3060–2760 (associated OH), 3440 (nonassociated OH), 1045 cm^{-1} (ether); λ_{max}^{EtOH} 253, 259, 269 (ε 410, 528, 293).

Anal. Calcd for $C_{19}H_{23}NO_5S_2 \cdot 2H_2O$: C, 50.99; H, 6.53; N, 3.13; S, 14.31. Found: C, 50.67; H, 6.04; N, 3.08; S, 14.12.

The material formed a sodium salt which was recrystallized from hot water: mp 235–237°.

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The Preparation and Biological Activities of Some Azonino- and Azecinoindoles and Benzazecines

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Various 1,2,3,4,5,6,7,8-octahydro-3-methylazonino[5,4-*b*]indoles, 1,2,4,5,6,7,8,9-octahydro-3-methyl-3H-azecino[5,4-*b*]indoles, and 1,2,3,4,5,6,7,8-octahydro-3-methyl-3-benzazecines of types **1–3**, respectively, have been prepared by reduction (with lithium and 1-methoxy-2-propanol in liquid ammonia) of the corresponding 2,3,5,6,11,11b-hexahydro-4-methylindolo[3,2-*g*]-1H-indolizinium iodides, 1,2,3,4,6,7,12,12b-octahydro-5-methylindolo[2,3-*a*]quinolizinium iodides, and 1,2,3,4,6,7-hexahydro-5-methyl-11bH-benzo[*a*]quinolizinium iodides of types **4–6**, respectively. Evidence is presented that the reduction involves an initial addition of two electrons. Biological activities are given for various members of the series **1–3**.

This paper records initial findings in a program for the synthesis and biological testing of compounds containing the azonino- and azecinoindole and benzazecine nuclei **1–3**, respectively. Our interest in members of this series arose from their structural relationship to corresponding benz- and indolindolizines and -quinolizines, including a number of alkaloids, which have been shown to possess interesting biological activities. These include potent pharmacodynamic effects on the central nervous system[1] and hypotensive[2] and amebicidal[3] activity.

We proposed to make compounds with the nuclei **1–3** through the metal–ammonia reduction of quaternary salts containing the corresponding moieties **4–6**, since, although the respective cations may, theoretically, undergo carbon–nitrogen cleavage in four distinct ways, the bond involving the benzylic carbon in the benzene series and the benzylic-like carbon in the indole series is expected to be the most susceptible.[4,5] We believed that salt formation on the indolic nitrogen would not affect the postulated cleavage of the indole cations of types **4** and **5** (R^1 = H), since such a reaction did not interfere with the selective cleavage of the allylic carbon–nitrogen bonds in agroclavine and elymoclavine methiodides.[6] After the completion of our work on the azonino- and azecinoindoles **1** and **2** (R = CH_3; R^1 = H), respectively (below), Wenkert and his colleagues[7] independently disclosed the preparation of the former compound, and Dolby and Booth[8] obtained the 2-hydroxy derivative of the amine **2** (R = CH_3) by methods similar to those reported here.

Azonino- and Azecinoindoles.—The bases **1** and **2** (R = CH_3) were prepared by the metal–ammonia reduction of the salts **4** and **5** (R = CH_3). Wenkert, *et al.*,[7] who have already reported the preparation of the first compound by the reduction of the salt **4** (R = CH_3; X = I) with an undisclosed amount of lithium and ethanol in liquid ammonia, assigned the structure from the elemental analysis and nmr spectra. The nmr evidence (NCH_3 singlet; no CCH_3 signal) while demonstrating that cleavage of the C–N bridgehead bond had occurred, did not definitely exclude an olefinic structure formed through Hofmann elimination. However, we observed no vinylic proton signals in the nmr spectrum, thereby confirming structure **1**. The presence in the nmr spectra of an NCH_3 singlet and the absence of CCH_3 and vinylic proton signals were similarly used to assign structures to all of the azonino- and azecinoindoles described in this paper. In a detailed examination of the preparation of **1** (R = CH_3), we found that lithium gave better conversion to the azoninoindole than sodium, and inclusion of 1-methoxy-2-propanol in the reaction medium gave improved yields with either metal. The beneficial effect of the alcohol may be due to its buffering action upon the reaction medium which, by ensuring that alkoxide rather than the more basic amide anion is formed by protonation of the reaction intermediates,[4] could reduce the incidence of side reactions of the Hofmann elimination type. The results of a comparative study of the reductive cleavage of the salt **4** (R = CH_3; R^1 = H;

(1) E. Schlittler in "The Alkaloids," Vol. VIII, R. H. F. Manske, Ed., Academic Press Inc., New York, N. Y., 1965, p 327, and references therein cited.

(2) J. F. Kerwin, C. P. Balant, and G. E. Ullyot in "Medicinal Chemistry," A. Burger, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, pp 561–567.

(3) E. F. Elslager, ref 2, pp 854–855.

(4) H. Smith, "Organic Reactions in Liquid Ammonia," Interscience Publishers, Inc., New York, N. Y., 1963, p 189, and references therein cited.

(5) E. Leete, *Chem. Ind.* (London), 692 (1960).

(6) S. Bhattacharji, A. J. Birch, A. Brack, A. Hofmann, H. Kobel, D. C. C. Smith, H. Smith, and J. Winter, *J. Chem. Soc.*, 121 (1962).

(7) E. Wenkert, S. Garratt, and K. G. Dave, *Can. J. Chem.*, **42**, 189 (1964).

(8) L. J. Dolby and D. L. Booth, *J. Org. Chem.*, **30**, 1550 (1964).

TABLE I

REDUCTION OF THE SALT **4** (R = CH_3; X = I) WITH ALKALI METALS IN LIQUID AMMONIA[a]

Metal (mg-atoms)[b]	1-Methoxy-2-propanol, mmoles	Yield, %[c]	Mp, °C
Li (2.16)	0	55	127–130
Li (2.16)	1.2	79	128–131
Na (2.16)	0	36	127–130
Na (2.16)	1.2	55	128-130.5

[a] Results obtained with 1 mmole of salt in each case. [b] Larger amounts gave lower yields. [c] Crude unrecrystallized product.

TABLE II

INDOLINDOLIZINIUM AND INDOLQUINOLIZINIUM SALTS

Compd	R	R^1	X	Crystn solvent[a]	Mp, °C	Formula	Yield, %[b]	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Halogen, % Calcd	Halogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found	Sulfur, % Calcd	Sulfur, % Found
4	CH_3	H	I	A–B, A	184.5–187.5	$C_{15}H_{19}IN_2$	78	50.9	50.6	5.4	5.7	35.8	35.5	7.9	7.6		
4	CH_3	CH_3	I	A	254–256	$C_{16}H_{21}IN_2$	73	52.18	52.45	5.75	5.82	34.5	34.3	7.6	7.2		
4	CH_3	H	*p*-$CH_3C_6H_4SO_3$	A	236–238	$C_{22}H_{26}N_2O_3S$	80	66.30	66.30	6.58	6.52			7.0	7.2	8.1	8.0
5	CH_3	CH_3	I	A	264–267 dec	$C_{17}H_{23}IN$	83	53.4	53.1	6.1	6.4	33.2	33.4	7.3	7.0		

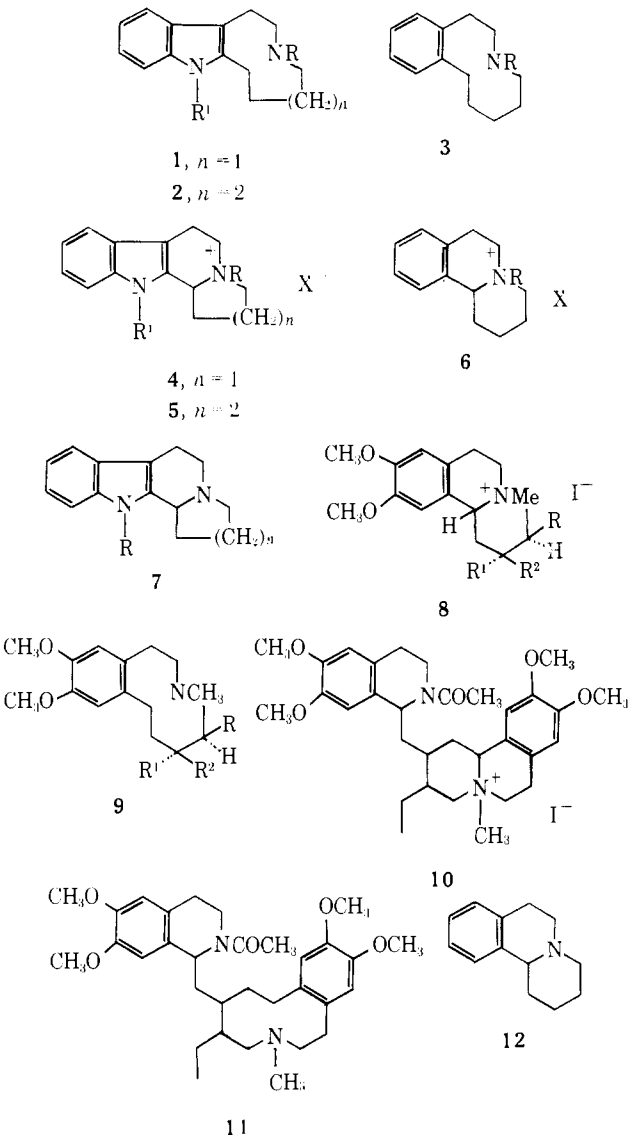
[a] A = methanol; B = ethyl acetate. [b] Before recrystallization.

TABLE III

FORMATION OF INDOLAZONINES, INDOLAZECINES, AND BENZAZECINES[a] BY LITHIUM–AMMONIA-ALCOHOL REDUCTION OF QUATERNARY AMMONIUM SALTS

Compd	R	R^1	R^2	X	Metal[b]	Product	R	R^1	R^2	Yield, %	Crystn solvent[c]	Mp, °C	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Chlorine, % Calcd	Chlorine, % Found	Nitrogen, % Calcd	Nitrogen, % Found
4[d]	CH_3	H		I	2.16	1	CH_3	H		58	C	130–132	$C_{15}H_{20}N_2$	78.9	79.1	8.8	8.9			12.3	12.3
						1[e]	CH_3	H			D, E	230–233[f]	$C_{15}H_{21}ClN_2$	68.0	67.8	8.0	8.2	13.4	13.4	10.6	10.3
4[d]	CH_3	CH_3		I	2.2	1[e]	CH_3	CH_3		33	F	198.5–201[f]	$C_{16}H_{23}ClN_2$	68.9	68.8	8.3	8.1	12.7	13.0	10.1	9.8
5[g]	CH_3	H		I	2.2	2	CH_3	H		45	C	95–97[f]	$C_{16}H_{22}N_2$	79.3	79.4	9.2	9.3			11.6	11.5
						2[e]	CH_3	H			A–B	145–150[f]	$C_{16}H_{23}ClN_2 \cdot 0.5H_2O$	66.8	66.6	8.4	8.4	12.3	12.5	9.7	9.8
5[d]	CH_3	CH_3		I	2.2	2[e]	CH_3	CH_3		31	F	221–223[f]	$C_{17}H_{25}ClN_2$	69.7	69.5	8.6	8.9	12.1	12.1	9.6	9.2
6[h]	CH_3			I	2.0	3	CH_3			86		25–28	$C_{14}H_{21}N$	82.7	82.6	10.4	10.1			6.9	6.7
						3[e]	CH_3				F	181–182	$C_{14}H_{22}ClN \cdot 0.2H_2O$	69.0	69.1	9.3	9.4	14.6	14.7	5.8	5.5
8[i]	H	H	H	I	2.0	9	H	H	H	84	C	58–60	$C_{16}H_{25}NO_2$	73.0	73.2	9.6	9.5			5.3	5.5
						9[e]	H	H	H		F	98–100	$C_{16}H_{26}ClNO_2 \cdot 0.5H_2O$	61.9	61.9	8.8	9.1	11.5	11.6	4.6	5.0
8[j]	$(CH_3)_2CHCH_2$	OH	H			9	$(CH_3)_2CHCH_2$	OH	H	35	C–G	136–138	$C_{20}H_{33}NO_3$	72.6	72.3	9.6	10.1			4.0	3.7
8[k]	$(CH_3)_2CHCH_2$	=O			2.2	9	$(CH_3)_2CHCH_2$	OH	H	10	C–F	134–136									
10[l]					2.2	11				88	G	174–176	$C_{32}H_{18}N_2O_5$	71.3	71.4	8.6	8.5			5.2	5.3
						11[e]						Amorphous[m]	$C_{32}H_{17}ClN_2O_5 \cdot H_2O$	64.8	65.2	8.3	8.5	5.9	6.3	4.7	4.6

[a] Salts of the bases are identified through footnotes. [b] Atoms of Li used/mole of substrate. [c] A = methanol, B = ethyl acetate, C = *n*-hexane, D = nitromethane, E = 2-propanol, F = acetone, G = ether. [d] This paper, Table II. [e] Hydrochloride. [f] Decomposition. [g] W. A. Reckhow and D. S. Tarbell, *J. Am. Chem. Soc.*, **74,** 4962 (1952). [h] S. Akaboshi, T. Kotsuma, and K. Achiwa, *Chem. Pharm. Bull.* (Tokyo), **8,** 14 (1960). [i] R. Child and F. L. Pyman, *J. Chem. Soc.*, 47 (1931). [j] A. Brossi, L. H. Chopard-dit-Jean, and O. Schnider, *Helv. Chim. Acta*, **41,** 1800 (1958). [k] A. Brossi, L. H. Chopard-dit-Jean, J. Wursch, and O. Schnider, *ibid.*, **43,** 583 (1960). [l] A. Ahl and T. Reichstein, *ibid.*, **27,** 373 (1944). [m] Not crystallized.



X = I) are given in Table I. Since optimum yields are obtained with little more than 2 equiv of metal, it would appear that, as in the reduction of agroclavine and elymoclavine methiodides,[6] reductive C–N cleavage is preferred to salt formation on the indolic nitrogen. Under our best conditions, the tosylate **4** (R = CH_3; R^1 = H; X = *p*-$CH_3C_6H_4SO_3$) gave only a 19% yield of the azoninoindole **1** (R = CH_3; R^1 = H), probably because of the low solubility of the substrate. No difficulty was found with the indolizinium salt **5** (R = CH_3; R^1 = H; X = I), which gave the corresponding azecinoindole in 45% yield, or with the indolic N-methylated derivatives of the salts **4** and **5** (R = CH_3; X = I), which gave the corresponding azonino- and azecinoindoles (isolated as the hydrochlorides) in yields of 33 and 31%, respectively. Reduction of the salts **4** (R = CH_2=$CHCH_2$ and CH≡CCH_2; R^1 = H; X = Br, and R = *p*-$CH_3OC_6H_4CH_2$; R^1 = H; X = Cl) and **5** (R = $C_6H_5CH_2$; R^1 = H; X = Br) gave as the only isolable product the indolizine **7** (R = H; *n* = 1) or the corresponding quinolizine, as appropriate, in yields of 99, 49, 75, and 78%, respectively, based on spectral examination of the total reaction product.

Lithium aluminum hydride selectively cleaves the bridgehead carbon–nitrogen bond in a quinolizinium salt related to **5**,[8] and hydrogenation over Adam's catalyst in methanol selectively cleaves the allylic carbon–nitrogen bond in agroclavine and elymoclavine methiodides.[9] In our hands, however, these procedures with the salt **4** (R = CH_3; R^1 = H; X = I) gave only intractable mixtures from which no azoninoindole was obtained.

Benzazecines.—Using the conditions established for the reduction of the salt **4** (R = CH_3; R^1 = H; X = I), **6** (R = CH_3; X = I) and its derivative **8** [R = $(CH_3)_2CHCH_2$; R^1,R^2 = H_2] gave high yields of the corresponding benzazecines of types **3** and **9**. These reductions proceed more rapidly than those of salts of types **4** and **5** (R = CH_3; R^1 = H; X = I) and may be performed by adding the metal piecemeal to the substrate and alcohol in liquid ammonia until a permanent blue color is developed.

On reduction, the salt **8** [R = $(CH_3)_2CHCH_2$; R^1 = OH; R^2 = H] gave the corresponding benzazecine of type **9**. With the ketone **8** [R = $(CH_3)_2CHCH_2$; R^1,R^2 = O] reduction of the carbonyl group accompanied carbon–nitrogen scission to give a low yield of the previously obtained alcohol **9** [R = $(CH_3)_2CHCH_2$; R^1 = OH; R^2 = H], but with N-acetylemetine methiodide **10**, scission was selective over reduction of the amide grouping and the complex benzazecine **11** was obtained in 88% yield.

Mechanism of the Metal–Ammonia Cleavage Reactions.—To obtain evidence upon possible mechanisms for the reductive scissions described here, we subjected the salt **6** (R = $C_6H_5CH_2$; X = Br) to the usual reductive treatment and obtained only the tricyclic base **12**. Ignoring steric considerations, this result suggests that reductive fission in this case involves a two-electron addition with removal of the benzylic group as the anion, since a one-electron addition would be expected to favor detachment of the more highly substituted benzylic carbon from nitrogen as a radical.[3] In support of this view, we have found that the salt **6** [R = 3,4-$(CH_3O)_2C_6H_3CH_2$; X = Br], in which the pendant benzyl group is substituted by a mesomerically electron-releasing *p*-methoxyl group, while giving mainly the base **12** also gave a small amount of the benzazecine **3** [R = 3,4-$(CH_3O)_2C_6H_3CH_2$] formed by scission of the bridgehead bond. The effect of a *p*-methoxyl group in inhibiting the reductive cleavage of benzyl alcohols *via* benzyl carbanions to toluene derivatives has been noted previously.[10]

The structure of the benzazecine **3** [R = 3,4-$(CH_3O)_2C_6H_3CH_2$] was confirmed by its preparation from the base **3** (R = CH_3) as follows. Initial von Braun degradation afforded the cyanamide **3** (R = CN) which, on reduction with lithium aluminum hydride, gave the base **3** (R = H), presumably through hydrolysis of an intermediate α,α-diamine. Alkylation with 3,4-dimethoxybenzyl bromide and triethylamine then gave the base **3** [R = 3,4-$(CH_3O)_2C_6H_3CH_2$], identical with the previously obtained sample.

Biological Activities.—The bases **1** and **2** (R = CH_3; R^1 = H)[11] and their indolic N-methyl derivatives are all active at a dose of 25 mg/kg ip in a rat diuretic

(9) J. P. Dickinson, J. Harley-Mason, and J. H. New, *J. Chem. Soc.*, 1858 (1964).

(10) See ref 4, p 160, and references cited therein.

(11) Each base was tested as the hydrochloride.

assay.[12] The second is the most active in this series showing good diuretic effects when given orally at 50 mg/kg both as to the volume of urine and the amount of sodium cation excreted. The benzazecine **3** (R = CH_3)[11] showed activity in the same test, at a dose of 25 mg/kg administered intraperitoneally, and its lower homolog **3** (R = H)[11] had antiinflammatory activity when administered intraperitoneally in a 10-mg/kg dose in an antiedema test in the rat.[13] In an *in vitro* test, the benzazecine **11** had the same order of amebicidal activity as emetine itself.[14]

Experimental Section[15]

Quaternary ammonium salts of general types **4–6** were made by the literature methods, or, when new, by reacting the appropriate base and halide or toluene-*p*-sulfonate in ethyl acetate or benzene for several hours, if necessary, under reflux. When solid, the new salts were purified by recrystallization from methanol or methanol–ether before metal–NH_3 reduction. The salts **4** (R = CH_2=$CHCH_2$ and CH≡CCH_2; R^1 = H; X = Br, and R = *p*-$CH_3OC_6H_4CH_2$; R^1 = H; X = Cl), **5** (R = $C_6H_5CH_2$; R^1 = H; X = Br), and **6** [R = $C_6H_5CH_2$ and 3,4-$(CH_3O)_2C_6H_3CH_2$; X = Br] were obtained only as gums and reduced as such. Characteristics of the new crystalline salts are given in Table II. Metal–NH_3 reduction is illustrated by two typical procedures. Bases 1 and **2** (R = R^1 = CH_3) and **2** (R = CH_3; R^1 = H) were purified by chromatography on neutral alumina. Data on the macrocyclic reduction products and/or their hydrochlorides are collected in Table III. Other necessary experimental details are included in the sequel.

2,3,5,6,11,11b-Hexahydro-11-methyl-1H-indolo[3,2-*g*]indolizine Hydrochloride.—2,3,5,6,11,11b-Hexahydro-1H-indolo[3,2-*g*]-indolizine (**7**, R = H; *n* = 1)[16] (8.5 g) in dimethylformamide (DMF) (200 ml) was stirred at 25° for 1 hr under nitrogen with sodium hydride (2.1 g of 50% w/w mineral oil dispersion). Methyl iodide (6.3 g) in DMF was added, and the mixture was stirred at 25° for 16 hr. The DMF was distilled and the residue, in $CHCl_3$, was washed with aqueous $KHCO_3$ then water and dried. The product was chromatographed on neutral alumina (activity III), elution with hexane–benzene and benzene giving the base **7** (R = CH_3; *n* = 1) as an oil (5.9 g): λ_{max} 229, 286, and 293 (sh) mμ (ε 34,100, 7000, and 6500); λ_{min} 252 mμ (ε 2200); nmr, 3-proton singlet at δ 3.6 (NCH_3). The **hydrochloride**, prepared by adding 2-propanol previously saturated with HCl to the base in ether melted at 244–247.5° (from acetone); λ_{max} 225.5, 284, and 292 (sh) mμ (ε 37,900, 7400, 6600); λ_{min} 250 mμ (ε 2200).

Anal. Calcd for $C_{15}H_{19}ClN_2$: C, 68.6; H, 7.3; Cl, 13.5; N, 10.7. Found: C, 68.6; H, 7.1; Cl, 13.7; N, 10.9.

1,2,3,4,6,7,12,12b-Octahydro-12-methylindolo[2,3-*a*]quinolizine Hydrochloride.[17]—Compound **7** (R = H; *n* = 2) [10.4 g, prepared analogously to **7** (R = H; *n* = 1)[16]] was converted by the foregoing methods to **7** (R = CH_3; *n* = 2): mp 54–61°; λ_{max} 229, 286, 291–294 (sh) mμ (ε 35,400, 6800, 6300); λ_{min} 252 mμ (ε 2300); nmr, 3-proton singlet at δ 3.7 (NCH_3). It was transformed then to the **hydrochloride**: mp 268–272° dec (from methanol–ethyl acetate); λ_{max} 226, 284.5, and 292.5 (sh) mμ (ε 38,600, 7300, 6400); λ_{min} 250 mμ (ε 2100).

Anal. Calcd for $C_{16}H_{21}N_2Cl$: C, 69.4; H, 7.65; Cl, 12.8; N, 10.1. Found: C, 69.3; H, 7.8; Cl, 13.2; N, 10.2.

1,2,3,4,5,6,7,8-Octahydro-3-methylazonino[5,4-*b*]indole (1, R = CH_3; R^1 = H).—Lithium (15 mg) was added with stirring to 2,3,5,6,11,11b-hexahydro-4-methylindolo[3,2-*g*]-1H-indolizinium iodide (0.35 g) and 1-methoxy-2-propanol (0.11 g) in liquid NH_3 (100 ml, distilled from lithium). Stirring was continued for 5 min when the blue color had been discharged and water (1 ml) was added. The NH_3 was removed under nitrogen and the residue was extracted with ether. The crude product (0.18 g), mp 128–131°, after two recrystallizations from *n*-hexane, gave the azoninoindole (0.13 g): mp 130–132°; λ_{max}^{KBr} 2.97, 3.55, 3.62, and 3.67 (sh) μ; λ_{max} 228.5, 285.5, 288–292 (plateau) mμ (ε 32,000, 7600, 7400); λ_{min} 254 mμ (ε 3300); nmr, 5-proton series of multiplets at δ 7.0–7.6 (aromatic CH and NH), 8-proton series of multiplets at 2.3–3.3 (CH_2 adjacent to aromatic nucleus or N), 3-proton singlet at 2.37 (NCH_3), 4-proton series of multiplets at 1.1–2.0 (CH_2).

The **hydrochloride**, formed with 2-propanol previously saturated at 0° with HCl and purified by recrystallization from nitromethane and 2-propanol, melted at 230–233° dec; λ_{max}^{KBr} 3.00, 3.85 μ; λ_{max} 224.5, 284.5, 291.5 mμ (ε 33,900, 7600, 6700); λ_{min} 247.5, 289.5 mμ (ε 1900, 6600).

1,2,3,4,5,6,7,8-Octahydro-3-methyl-3-benzazecine (3, R = Me).—Lithium (112 mg) was added in small pieces with stirring to 1,2,3,4,6,7-hexahydro-5-methyl-11bH-benzo[*a*]quinolizinium iodide (2.6 g) and 1-methoxy-2-propanol (0.88 g) in liquid NH_3 (300 ml). After addition of the last piece, the solution became intensely blue. After 3 min the color was discharged by a few drops of water, the ammonia was evaporated by gentle warming, water was added, and the mixture was extracted with ether. The product, a low-melting solid (1.4 g), distilled at 130° (0.1 mm) to give the benzazecine (1.2 g): mp 25–28°; λ_{max}^{film} 3.45, 3.65, 6.70, and 6.75 μ; λ_{max}^{EtOH} 266.5 and 273.5 mμ (ε 460, 450); nmr, 4-proton singlet at δ 7.11 (aromatic CH), 8-proton series of multiplets at 2.10–3.00 (CH_2 adjacent to the aromatic nucleus or N), 3-proton singlet at 2.04 (NCH_3), 6-proton series of multiplets 1.20–1.90 (CH_2), no signal for methyl attached to methylene in the 0.8–1.2 region, or for vinyl protons in 4.0–5.5 region.

1,2,3,4,5,6,7,8-Octahydro-3-benzazecine (3, R = H).—The benzazecine **3** (R = CH_3) (5.5 g) was kept in ether with cyanogen bromide (5 g) for 15 hr at room temperature. The mixture was added to ice–water and extracted with ether. The product was refluxed with diethylamine (50 ml) in benzene (100 ml) for 14 hr. The cooled solution was filtered to give diethylamine hydrobromide (1.8 g). The filtrate was diluted with ether and washed with 2 *N* HCl and water. The organic layer was dried and evaporated to give a gum (1.8 g), λ_{max}^{film} 4.55 μ. An aliquot (1.5 g) was refluxed overnight with lithium aluminum hydride (2 g) in ether (100 ml). Water was added, the mixture was extracted with ether, and the ether extracts were extracted with 2 *N* HCl. Basification of the aqueous layer with solid $NaHCO_3$ gave a base (1 g) which was distilled at 200° (bath, 0.1 mm) to give the benzazecine (0.73 g): λ_{max} 266 and 273.5 mμ (ε 830, 850); λ_{max}^{film} 3.35, 3.45, 3.52 μ; nmr, 4-proton singlet at δ 7.29 (aromatic CH), 8-proton series of multiplets at 2.6–3.2 (CH_2 adjacent to aromatic nucleus or N), 6-proton series of multiplets at 1.0–2.0 (CH_2).

Anal. Calcd for $C_{13}H_{19}N$: C, 82.5; H, 10.1; N, 7.4. Found: C, 82.2; H, 9.9; N, 6.9.

3-(3,4-Dimethoxybenzyl)-1,2,3,4,5,6,7,8-octahydro-3-benzazecine [3, R = 3,4-$(CH_3O)_2C_6H_3CH_2$]. A.—The benzquinolizine **12** (2.8 g) was refluxed with 3,4-dimethoxybenzyl chloride (3.5 g) in benzene (5 ml) for 2 hr. On cooling the supernatant solution was decanted from the gummy residue (3.2 g) which was dissolved in liquid NH_3–1-methoxy-2-propanol (300 ml:0.85 g). Lithium was added piecemeal with stirring until the solution became permanently blue, and the color was discharged with several drops of water. The product, in ether, was extracted with 2 *N* HCl, and the acidic extracts were basified with aqueous NH_3. Treatment of the product with 2-propanol (20 ml, previously saturated at 0° with HCl) precipitated the hydrochloride of **12** (1.15 g) which was filtered. Evaporation of the filtrate gave a residue which was dissolved in methanol and purified by preparative thin layer chromatography on silica gel plates[18] (thickness 1 mm) using benzene saturated with NH_3 as developing solvent to give the benzazecine (82 mg): mp 55–57° (from hexane); nmr, 7-proton series of multiplets at δ 6.4–7.4 (aromatic CH), two 3-proton singlets at 3.61 and 3.88 (OCH_3),

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(15) Melting points were determined in capillary tubes and are uncorrected. Ultraviolet absorption spectra were determined in 95% ethanol. Proton magnetic resonance spectra were measured on the Varian A-60 spectrometer using 5–10% solutions in $CDCl_3$ containing tetramethylsilane (TMS) as internal reference standard. Chemical shifts are expressed in δ units and should be accurate to ±0.1 ppm.

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2-proton singlet at 3.38 ($NCH_2C_6H_5$), 8-proton series of multiplets at 2.2–3.0 (CH_2 adjacent to aromatic nucleus or N), and 6-proton series of multiplets at 1.2–2.0 (CH_2).

Anal. Calcd for $C_{22}H_{29}O_2N$: C, 77.8; H, 8.6; N, 4.1. Found: C, 76.9; H, 8.8; N, 3.9.

B.—The benzazecine **3** (R = H) (0.12 g) was refluxed for 16 hr with triethylamine (0.3 g) and 3,4-dimethoxybenzyl chloride (0.3 g) in $CHCl_3$ (5 ml). Distillation of the product at 170° (bath, 0.1 mm) gave the benzazecine **3** [R = 3,4-$(CH_3O)_2$-$C_6H_3CH_2$], mp 53–56°, undepressed by the sample prepared as in A.

Reductive Fission of Salt 6 (R = $C_6H_5CH_2$; X = Br). Lithium (56 mg) was added piecemeal with stirring to the salt (1.5 g) in liquid NH_3 (200 ml) containing 1-methoxy-2-propanol (0.43 g). After 5 min the blue color was discharged with a few drops of water, and ether and water were added. The oily product in ether was treated with isopropyl alcohol previously saturated with HCl at 0°, and the resulting suspension was filtered to give the **hydrochloride** of base **12**, identical in melting point, infrared absorption spectrum, and thin layer chromatographic behavior (on silica gel using NH_3-saturated benzene) with an authentic sample.

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Synthesis of Methylenetetrahydrofolic Acid Analogs[1]

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The synthesis of analogs of N^5,N^{10}-methylenetetrahydrofolic acid is described. These compounds are 2-substituted 1-*p*-carbethoxyphenylimidazolidines and 3-substituted 2-*p*-carbethoxyoctahydroimidazo[1,5-*a*]-pyrazines, structural modifications of the cofactor involved in the synthesis of thymidylic acid. The 5′-uracil derivatives are analogs of the proposed intermediate in the biosynthesis of thymidylic acid. The inhibitory effects of these compounds on dihydrofolate reductase and thymidylate synthetase are described.

The role of folate coenzymes in biochemical pathways has been amply reviewed by many investigators.[2] Several aspects of folate utilization have been of particular interest in the design of agents effective in the treatment of cancer, *e.g.*, aminopterin and amethopterin. Tetrahydrofolic acid was recognized in 1957 as an essential metabolite in the synthesis of thymidine 5′-monophosphate.[3] The unique character of the one-carbon transfer, a reductive methylation, stimulated further interest in this pathway.

If the limiting factor in deoxyribonucleic acid synthesis and ultimately cell division is the availability of thymidine 5′-triphosphate,[4] then an obvious approach to potential anticancer agents is through the inhibition of thymidine 5′-triphosphate formation. One such step in the sequence is the synthesis of thymidine 5′-monophosphate. Several studies on the mechanism of the one-carbon transfer to deoxyuridine 5′-monophosphate (dUMP) have demonstrated that tetrahydrofolic acid, the reducing agent, is converted to dihydrofolic acid[5] *via* a hydrogen (hydride?) transfer in the intermediate complex to give the product, thymidine 5′-monophosphate. The enzyme thymidylate synthetase, isolated from microbial and mammalian sources,[5,6] catalyzes the reductive methylation of deoxyuridine 5′-monophosphate by transfer of a methyl group from N^5,N^{10}-methylenetetrahydrofolic acid. The kinetics of the reaction support the view that a binary complex is formed between the enzyme and the cofactor with subsequent formation of a ternary intermediate with the substrate (dUMP).[7] After transfer of the methyl, the binary enzyme–dihydrofolic acid complex dissociates. Regeneration of tetrahydrofolic acid is mediated by nicotinamide–adenine dinucleotide phosphate (NADPH) reduction of dihydrofolic acid in the presence of dihydrofolic acid reductase.

Inhibition of the latter step in the sequence by aminopterin and amethopterin has been demonstrated as the site of action for these drugs.[8] Direct inhibition of thymidylate synthetase has been reported for 5-fluoro- and 5-trifluoromethyl-2′-deoxyuridine 5′-monophosphate[6b,7c] which can be termed, respectively, "substrate" and "product" inhibitors. The proposed formation of a binary complex and the kinetics of the enzymatic reaction suggest the feasibility of inhibition by analogs of the cofactor, N^5,N^{10}-methylenetetrahydrofolic acid.

The design of cofactor inhibitors of thymidylate synthetase is not new. Kisliuk[9] reported growth

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