RESULTS AND DISCUSSION

The administration of cobaltous chloride or sodium cobaltinitrite to pregnant mice on Day 10 or 11 of gestation with physiological saline caused cleft palates in the fetus, the higher incidence being associated with the earlier cobalt challenge. Because molar cobalt equivalents elicited qualitatively comparable palate defects, the role of the metallic ion in causing this malformation in mice is established beyond doubt. The administration of either cobaltous chloride or sodium cobaltinitrite on Day 10 or 11 (Groups F, G, and H) with cortisone (Days 11–14) inhibited cleft palates significantly ($p = <0.005$) when compared to the incidence attained with cortisone alone (Groups C, D), with the greater protection afforded on the earlier day of challenge. Group O, nickel chloride (Day 10) and saline (Days 11–14), was devoid of clefts. Although a significant inhibition ($p = <0.005$) was noted when nickel chloride (Day 10) was administered with cortisone (Days 11–14, Group N), it was not as marked as that observed with either cobalt compound and cortisone (Groups F, G, H). No alteration in the incidence of cortisone-induced cleft palate ($p = <0.5$) was noted when the steroid was administered on Days 11–14 (Group C) or 10–13 (Group D).

Several aspects of the ionic hormonal precursor hypothesis have been verified by this study:

1. The ability of cobalt ion to induce cleft palates in mice in the manner of cortisone and to prevent this malformation when caused by the steroid has been confirmed with cobaltous chloride and sodium cobaltinitrite.
2. The younger the fetus at the time of cobalt challenge, the more dramatic are the responses of cleft palate induction and inhibition in the absence and presence of the steroid, respectively. Age, however, did not influence the incidence of cleft palate caused by cortisone. Thus, from the evolutionary standpoint, one would anticipate a greater biological response to the "hormone precursor"—cobalt—than to that of its more recent counterpart—cortisone—which was indeed the case.
3. Cobalt displays greater ionic specificity than nickel because the former both induces and inhibits cleft palate alone and in the presence of the steroid, while the latter only possesses an inhibitory capability which implies a different mechanism of action at the palatine tissue level.

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(6) D. E. Mann, Jr., R. F. Gautieri, and G. Kasirsky, *Lancet*, **1968-I**, 699.

(7) H. S. Harpel, Jr. and R. F. Gautieri, *J. Pharm. Sci.*, **57**, 1590 (1968).

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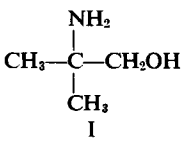
N-Substituted-2-amino-2-methyl-1-propanols as Potential Antitumor Agents

JOHN H. BILLMAN, FRED KOEHLER, and RALPH MAY

Abstract □ Twenty-seven Schiff-base derivatives of 2-amino-2-methyl-1-propanol have been prepared and submitted for antitumor testing. Thirteen *N*-substituted-2-amino-2-methyl-1-propanols were prepared by the reduction of the above Schiff bases. These also were submitted for antitumor testing.

Keyphrases □ 2-Amino-2-methyl-1-propanols, *N*-substituted—synthesis □ Schiff-base derivatives—2-amino-2-methyl-1-propanols □ Antitumor activity—Schiff bases □ IR spectrophotometry—structure □ NMR spectroscopy—structure

2-Amino-2-methyl-1-propanol (I), is an effective antitumor agent (1).

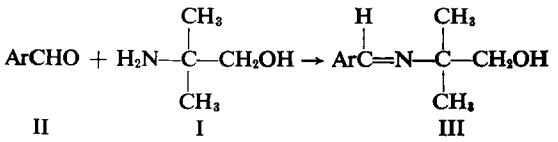


I

Few *N*-substituted derivatives of this compound have been made and apparently none have been tested for antitumor activity. The authors' interest is centered in modifying the structure of 2-amino-2-methyl-1-propanol (I)[1] in the hopes of improving the effectiveness of this drug.

It may be assumed that due to the reactivity of the amino group in 2-amino-2-methyl-1-propanol (I) a reasonable percentage of the applied dose may never reach the tumor site. One means of averting this problem is to protect the amino group in a manner that will allow the blocking group to be removed selectively at the tumor site. Ideally, this might be expected to dramatically improve the therapeutic indexes. The approach that was selected in this project was to form Schiff-base derivatives of the type shown in Scheme I.

[1] 2-Amino-2-methyl-1-propanol is sometimes referred to as AMP.

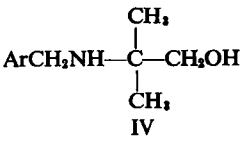


II I III

Scheme I

The rationale behind this choice was twofold. Since tumor cells are generally more acidic than normal cells, one might expect the Schiff bases to be preferentially hydrolyzed at the tumor site with regeneration of the active amine since compounds of this type are readily hydrolyzed in an acid medium. Since certain aromatic aldehyde derivatives have been shown to be very active antineoplastic agents (2), it was felt that similar 2-amino-2-methyl-1-propanol (I) derivatives (Table I) would be equally effective. The activity of the proposed Schiff bases could be due to the molecule as a whole or through the liberation of active aldehydes and/or 2-amino-2-methyl-1-propanol (I) at the tumor site.

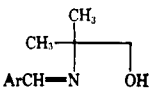
A second phase of this work was to prepare *N*-benzyl-2-amino-2-methyl-1-propanols (IV) (Table II) to



IV

determine if these derivatives of 2-amino-2-methyl-1-propanol (I) might possess greater antitumor activity. These compounds can be prepared from the correspond-

Table I—Benzylidine Derivatives of 2-Amino-2-methyl-1-propanol



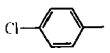
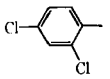
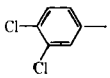
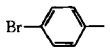
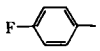
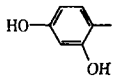
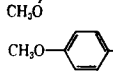
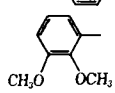
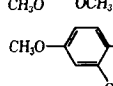
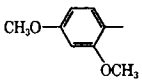
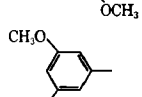
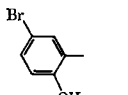
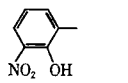
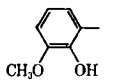
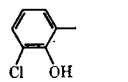
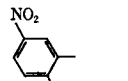
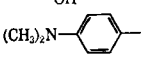
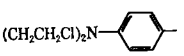
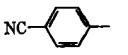
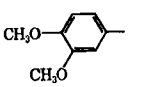
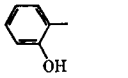
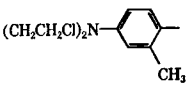
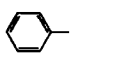
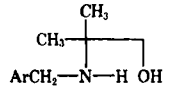
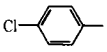
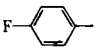
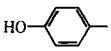
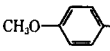
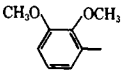
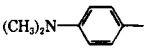
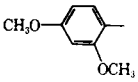
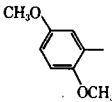
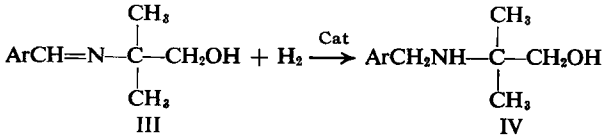
Compd.	Ar	Formula	Yield (pure), %	M.p., °C.	%N Calcd.	%N Found	Method
1		$C_{11}H_{14}ClNO$	66.0	66–68	6.62	6.74	B
2		$C_{11}H_{14}ClNO$	84.0	63–64	6.62	6.88	B
3		$C_{11}H_{13}Cl_2NO$	94.4	96–98	5.69	5.55	B
4		$C_{11}H_{14}Cl_2NO$	51.4	171.5–173	4.96	5.03	B
5		$C_{11}H_{14}BrNO$	92.8	124–125 (0.7 mm.)	5.47	5.64	B
6		$C_{11}H_{14}BrNO$	69.9	93–94	5.47	5.57	B
7		$C_{11}H_{14}BrNO$	96.5	64.5–66	5.47	5.50	A
8		$C_{11}H_{14}FNO$	65.7	78–80	7.17	7.08	B
9		$C_{11}H_{14}FNO$	71.4	63–65	7.17	7.10	B
10		$C_{11}H_{14}FNO$	83.4	80–81.5	7.17	7.27	B
11		$C_{11}H_{15}NO_2$	83.3	158.5 D	6.69	6.53	B
12		$C_{12}H_{17}NO_2$	92.6	66–67.5	6.76	6.66	B
13		$C_{12}H_{17}NO_2$	97.6	119–121 (0.5 mm.)	6.76	6.76	A
14		$C_{12}H_{17}NO_2$	99.0	53.5–55	6.76	6.70	A
15		$C_{13}H_{19}NO_3$	79.1	138–141 (0.3 mm.)	5.92	5.95	B
16		$C_{13}H_{19}NO_3$	87.0	74.5–76	5.92	5.90	B
17		$C_{13}H_{19}NO_3$	91.5	72–73	5.92	5.87	B
18		$C_{13}H_{19}NO_3$	78.8	164 (1.0 mm.)	5.92	5.64	B
19		$C_{11}H_{14}BrNO_2$	92.8	111–113	5.15	5.25	A
20		$C_{11}H_{14}N_2O_4$	91.4	144–146	11.76	12.08	A
21		$C_{12}H_{17}NO_3$	87.9	119–121	6.27	6.33	A
22		$C_{11}H_{13}Cl_2NO_2$	92.4	148–150	5.34	5.37	A
23		$C_{11}H_{14}N_2O_4$	92.6	208–209.5	11.76	11.83	A
24		$C_{13}H_{20}N_2O$	71.5	112–114	12.71	12.54	B
25		$C_{15}H_{22}Cl_2N_2O$	77.0	78.5–79.5	8.83	9.03	B
26		$C_{12}H_{14}N_2O$	13.1	148–151 (2.0 mm.)	13.85	13.48	B
27		$C_{12}H_{19}NO_3$	83.3	97–98.5	5.91	5.92	B
28		$C_{11}H_{15}NO_2$	79.5	65–67	7.25	7.14	B
29		$C_{16}H_{24}Cl_2N_2O$	67.5	87–89	8.46	8.39	B
30		$C_{11}H_{15}NO$	75.5	67–69	7.90	7.82	B

Table II—*N*-Substituted Benzyl-2-amino-2-methyl-1-propanol



Compd.	Ar	Formula	Yield (pure), %	M.p., °C.	%N Calcd.	%N Found
1		$C_{11}H_{16}ClNO$	90.4	71.5–73	6.56	6.37
2		$C_{11}H_{16}ClNO$	74.8	103–104	6.56	6.63
3		$C_{11}H_{16}FNO$	61.4	60–62	7.10	7.24
4		$C_{11}H_{16}FNO$	77.3	51–53	7.10	7.24
5		$C_{11}H_{16}FNO$	75.2	77–79	7.10	7.30
6		$C_{11}H_{15}ClNO_2$	86.2	201.5–203	6.04	6.01
7		$C_{11}H_{17}NO_2$	36.0	64–65.5	7.17	7.29
8		$C_{12}H_{19}NO_2$	73.3	138–142 (1.5 mm.)	6.69	6.82
9		$C_{12}H_{19}NO_2$	91.2	57–59	6.69	6.68
10		$C_{12}H_{19}NO_2$	93.9	58–60	6.69	6.70
11		$C_{12}H_{22}ClNO_3$	71.1	150–152	5.08	5.11
12		$C_{13}H_{22}N_2O$	71.4	75–77	12.60	12.82
13		$C_{13}H_{21}NO_3$	67.5	59.5–61.5	5.85	6.06
14		$C_{13}H_{21}NO_3$	71.2	59–61	5.85	5.47

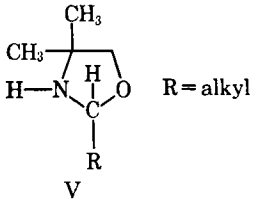
ing Schiff bases by catalytic hydrogenation as shown in Scheme II.



Scheme II

DISCUSSION

The Schiff bases were prepared by the customary procedures. Twenty-seven of these compounds were synthesized and sent to the Cancer Chemotherapy National Service Center for antitumor screening. Thirteen of the corresponding amines were prepared and also sent for testing. A question arose during the course of this work as to the structure of the products formed in Scheme I. It is well known that the condensation of aldehydes with β-amino alcohols yield either heterocyclic molecules (oxazolidines in this case), Schiff bases, or both (3, 4). A recent article (5) described the condensation of 2-amino-2-methyl-1-propanol (I) with various aliphatic aldehydes. The products reported by Clapp *et al.* were the oxazolidines (V).



These investigators did a thorough analysis of their products. However, the structural assignment was based to a large extent on a triplet absorption at 1,080–1,200 cm.$^{-1}$ in the IR spectra and a ring CH proton signal at 5.6 τ in the NMR spectra. In the present instance the assignment of the Schiff-base structure (III) was made on the basis of an absorption at 1,650 cm.$^{-1}$ in the IR region and the azomethine proton signal at 1.5 τ in the NMR spectra. In these NMR spectra the azomethine proton always integrated as one proton.

Antitumor testing data have only been reported for eight of these Compounds 1, 3, 5, 7, 14, 23, 25, 27. One of these, Compound 25, has shown 97% inhibition of Walker 256 carcinoma in preliminary testing.

General Procedures for Condensation of 2-Amino-2-methyl-1-propanol with Benzaldehydes—*Method A*—Equimolar amounts of 2-amino-2-methyl-1-propanol and the benzaldehyde were dissolved in sufficient dry benzene to form an approximately 1 *M* solution with respect to each reactant. The mixture was refluxed for 1 hr. The water formed by the reaction was then removed by azeotropic distillation until the theoretical amount was obtained or until no further azeotrope was observed being produced. The remainder of the solvent was then removed under reduced pressure, and the residual crude product was purified by recrystallization (from either acetonitrile, ethanol, 1-propanol, or 2-propanol), distillation, sublimation, or column chromatography (neutral alumina column—Woelm, activity Grade I).

Method B—Equimolar amounts of 2-amino-2-methyl-1-propanol and the benzaldehyde were dissolved in separate portions of dry methanol. Sufficient solvent was used to give an approximately 1 *M* solution of both reactants. The amino alcohol was then added dropwise to the benzaldehyde over a period of 1 hr. During this time the reaction flask was warmed and the mixture was stirred. After complete addition the reaction mixture was refluxed for 1 hr. Following this the solvent and the water formed in the reaction were removed under reduced pressure, and the residual crude product was purified by recrystallization (as in Method A), distillation, sublimation, or column chromatography (neutral alumina column—Woelm, activity Grade I).

General Procedure for the Reduction of Schiff Bases—The Schiff base was dissolved in ethanol in which was suspended platinum oxide. The mixture was placed on a hydrogenator[2] at an initial pressure of approximately 45 p.s.i., and after the theoretical uptake of hydrogen had occurred it was removed. The ethanol was removed under reduced pressure and the residual crude product was purified by recrystallization (as in Method A), distillation, or sublimation.

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[2] Paar.