**Table I—Dilution Factors [a]**

| Drug | Conventional Tablets | Sustained-Action Tablets |
|---|---|---|
| Theophylline | 733.3 | 1000 |
| Ephedrine hydrochloride | 627.3 | 941 |
| Phenobarbital | 667 | 1000 |

[a] The theophylline factor includes 1.100, the ratio of the molecular weights of hydrous and anhydrous theophylline. The ephedrine hydrochloride factor includes 0.941, the ratio of the equivalent weights of the hydrochloride and sulfate salts.

standard preparation, respectively; $T$ and $S$ are the weights, in milligrams, of the average tablet and of the portion of the tablet taken for assay, respectively; and $F$ represents the factors from dilutions (Table I).

## RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms for a conventional tablet formulation and a placebo mixture in which a reagent peak appears at ~2.3 min due to iodate, at ~3.5 min due to theophylline, at ~6.0 min due to phenobarbital, and at ~9.4 min due to butabarbital; benzaldehyde, from ephedrine, has a retention time of ~11.7 min. The chromatograms show no interference from the excipients and the oxidation products of propylene glycol, formaldehyde, and acetaldehyde.

**Table II—Precision and Recovery Data**

| Trial | Percent Recovery: Theophylline | Ephedrine Hydrochloride | Phenobarbital |
|---|---|---|---|
| | Conventional Tablets | | |
| 1 | 98.4 | 99.2 | 97.8 |
| 2 | 99.4 | 97.6 | 100.8 |
| 3 | 98.1 | 98.0 | 96.6 |
| 4 | 98.7 | 100.1 | 99.6 |
| 5 | 100.3 | 99.2 | 100.0 |
| 6 | 98.4 | 97.5 | 96.3 |
| Mean | 98.9 | 98.6 | 98.5 |
| *RSD*, % | 0.83 | 1.07 | 1.91 |
| | Sustained-Action Tablets | | |
| 1 | 98.6 | 98.3 | 98.1 |
| 2 | 101.4 | 100.7 | 100.3 |
| 3 | 99.3 | 100.0 | 99.7 |
| 4 | 100.7 | 100.0 | 99.7 |
| 5 | 100.0 | 101.4 | 100.3 |
| 6 | 100.0 | 99.3 | 99.7 |
| Mean | 100.0 | 100.0 | 99.6 |
| *RSD*, % | 0.99 | 1.08 | 0.81 |

**Table III—Recovery Data at 80 and 120% Label Claim**

| Drug | Percent Found: 80 | 120 |
|---|---|---|
| | Conventional Tablets | |
| Theophylline | 82.2, 81.9 | 117.8, 119.5 |
| Ephedrine hydrochloride | 82.4, 82.6 | 120.3, 123.1 |
| Phenobarbital | 79.7, 79.0 | 121.4, 118.8 |
| | Sustained-Action Tablets | |
| Theophylline | 80.7, 80.7 | 117.9, 118.8 |
| Ephedrine hydrochloride | 79.4, 79.4 | 118.4, 118.6 |
| Phenobarbital | 79.1, 79.7 | 119.5, 120.3 |

The determination of ephedrine as benzaldehyde after periodate oxidation was shown to be stability indicating by Chafetz (4). Omission of periodate from the system had no effect on the retention times or response of theophylline, phenobarbital, or butabarbital. Ephedrine was moved to a retention time of ~20 min; however, its response was decreased by a factor of >200. Ephedrine can be visualized only by changing the detector to ~260 nm and using an increased concentration or lower attenuation.

The UV detection of phenobarbital and butabarbital at 241 nm is due to the monoanion form of the ureide ring, the unionized form having little absorbance in the near UV; hence, the incorporation of pH 7.8 buffer in the mobile phase. This mobile phase provides sufficient alkalinity to be in the $pKa_1$ range for the barbiturates and for a rapid reaction rate of periodate with ephedrine. (The apparent pH of the mobile phase is 8.3.)

Precision and recovery data for conventional and sustained-action theophylline, ephedrine hydrochloride, and phenobarbital tablets are shown in Tables II and III. Calculation of resolution factors between adjacent peaks provides values greater than 5. Peak response ratios *versus* concentrations for each component to the internal standard were rectilinear. Recovery was determined by adding known amounts of the drugs to the excipients, which included starch, lactose, stearic acid, and polyethylene glycol 6000 for conventional tablets and starch USP, lactose USP, stearic acid powder, polyethylene glycol 6000, guar gum, methylcellulose USP, sugar powder, ethylcellulose NF, magnesium stearate USP, carnauba wax, D&C Red No. 30 lake, and D&C Yellow No. 10 lake for sustained-action tablets.

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# Catecholamine Analogs as Potential Antitumor Agents II


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Received September 3, 1980, from the *Drug Design and Chemistry Section, Laboratory of Medicinal Chemistry and Biology, Developmental Therapeutics Program, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.* Accepted for publication December 2, 1980.



**Abstract** □ Polyhydroxybenzylamine derivatives related to dopamine were synthesized and shown to have activity against murine P-388 lymphocytic leukemia. The 3,4,5-trihydroxy- and 2,3-dihydroxybenzylamine hydroiodides were active, as were several other catechol derivatives capable of *o*-quinone formation.



**Keyphrases** □ Catecholamine analogs—polyhydroxybenzylamine hydroiodides synthesized and evaluated for antitumor activity, mice □ Structure–activity relationships—catecholamine analogs synthesized and tested for antitumor activity, mice □ Antitumor activity—catecholamine analogs synthesized and evaluated for antitumor activity, mice


Phenalkylamines (1–4) and pyridine derivatives (5) possessing vicinal hydroxyl groups have antitumor activity against *in vivo* murine tumor models and related *in vitro* systems. A previous investigation (4) explored structure–




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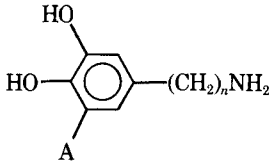
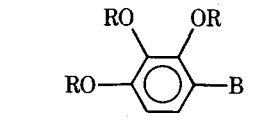
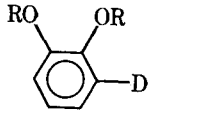


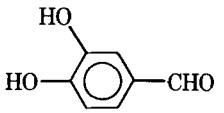
I: A = H, $n$ = 1
II: A = H, $n$ = 2
III: A = OH, $n$ = 2



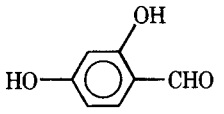
VI: B = $CH_2NH_2$, R = $CH_3$
VII: B = $CH_2\overset{+}{N}H_3I^-$, R = H
VIII: B = $COCH_3$, R = H
IX: B = $COC_9H_{19}$, R = H



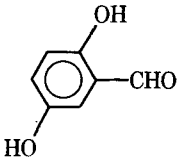
X: D = $CH_2NH_2$, R = $CH_3$
XI: D = $CH_2\overset{+}{N}H_3I^-$, R = H
XII: D = COOH, R = H



XIII



XIV



XV

activity studies among catecholamine analogs, with emphasis on the P-388 leukemia activity of phenethylamine and benzylamine compounds. In that study, the benzylamine analog (I) of dopamine (II) was active against P-388 leukemia. The trihydroxy compound, 5-hydroxydopamine (III), also was active. The current study extended this work to additional benzylamine derivatives including two trihydroxy analogs.

## RESULTS AND DISCUSSION

3,4,5-Trimethoxybenzylamine (IV) was prepared by two routes to determine the advantages of catalytic *versus* chemical reduction as synthetic procedures in the benzylamine series (Scheme I). Reduction of the amide with sodium acetoxyborohydride (6) produced IV in a 50% yield. Catalytic reduction of the nitrile in chloroform by the method of Secrist and Logue (7) gave a somewhat better yield of IV (75%) and is the method of choice. Cleavage of the methoxyl groups with hydroiodic acid (8) yielded the target compound 3,4,5-trihydroxybenzylamine as the hydroiodide (V). Several isomeric di- and trihydroxybenzylamines were prepared previously as either free bases or hydrochloride salts (9). The synthesis of 2,3,4-trihydroxybenzylamine hydroiodide (VII) was accomplished by the borohydride method, while 2,3-dihydroxybenzylamine hydroiodide (XI) was prepared in good yield by catalytic reduction of the nitrile followed by hydroiodic acid treatment.

The antitumor results are shown in Table I. The compounds (I–III) on which the present study is based were previously shown to be active against P-388 leukemia (4). The target compounds, V and XI, were determined to be active, but VII gave T/C values[1] of only 123 and 121%. The corresponding acetophenone (VIII), however, was active, and the activity of 3,4-dihydroxybenzaldehyde (XIII) was confirmed.

The generalized structure–activity relationships described previously for the catecholamines (4) continue to apply in the benzylamine series. Positioning of vicinal hydroxyl groups in the benzylamine series such that oxidation to *ortho*-derivatives might occur provides active materials (I, V, VIII, XI, and XIII). Dihydroxy compounds that cannot be oxidized to a quinoid form (XIV) or can be oxidized only to a *p*-quinone (XV) are inactive. As noted previously (4), dihydroxybenzoic acid (XII) analogs are not active. The addition of an apparent excess of lipophilic character converts an active compound (VIII) to an inactive one (IX).

2,3,4-Trihydroxybenzylamine (VII) was expected to be active against P-388 leukemia but was not. Two good P-388 dose–response experiments employing five doses each (range 600–50 mg/kg) gave values that approached but did not exceed a T/C value of 125%. Compound VII showed more toxicity than the other analogs, with the doses at 600, 400, and 200 mg/kg being toxic in each experiment.

[1] Percent T/C = (treated survival to control survival) × 100%.

**Table I—P-388 Lymphocytic Leukemia Antitumor Activity [a]**

| Compound | Optimum Dose, mg/kg/day | T/C, % | T − C, g |
|---|---|---|---|
| I | 700 | 158 | −2.9 |
| II | 600 | 168 | −1.7 |
| III | 400 | 168 | −3.6 |
| V | 600 | 157 | −3.4 |
| VII | 50 | 123 | −0.5 |
| VIII | 400 | 137 | −2.1 |
| IX | 200 | 105 | −2.6 |
| XI | 600 | 149 | −1.9 |
| XII | 100 | 104 | +0.5 |
| XIII | 400 | 138 | −0.5 |
| XIV | 100 | 102 | −0.6 |
| XV | 100 | 114 | −1.4 |

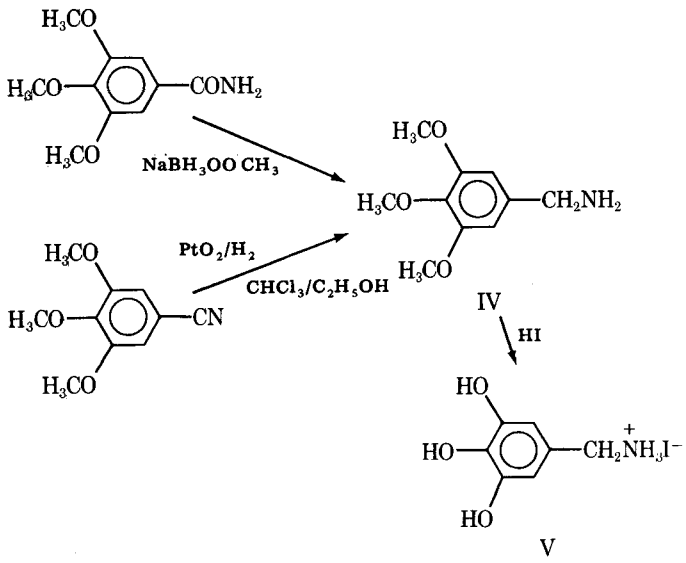
[a] See *Experimental* for conditions and definitions.

## EXPERIMENTAL[2]

**3,4,5-Trimethoxybenzylamine (IV)**—*Method A*—3,4,5-Trimethoxybenzamide (3.6 g, 18 mmoles) and sodium borohydride (3.1 g, 82 mmoles) were suspended in 40 ml of dried *p*-dioxane. To the suspension was added acetic acid (4.8 g, 80 mmoles) in 20 ml of dried *p*-dioxane over 20 min at 10°. The resulting mixture was refluxed for 3 hr. After cooling, water was added to decompose the excess borohydride, and the mixture was evaporated to dryness under reduced pressure. The residue was stirred in 100 ml of chloroform and filtered, and the filtrate was saturated with hydrogen chloride gas. A white hydrochloride was produced after evaporation, and it was dissolved in a minimum amount of water and extracted once with ethyl acetate.

The aqueous layer was made basic with 10% NaOH and saturated with sodium chloride. The mixture was extracted twice with ether. The ether extracts were combined, dried over sodium sulfate, and evaporated to dryness to give 1.8 g (50%) of an oil. The NMR spectrum indicated that IV was pure; NMR (deuterochloroform): 1.68 (s, 2H, $NH_2$), 3.9 (m, 11H, $CH_3$ and $CH_2$), and 6.65 (s, 2H, aromatic). This product was used without further purification for the next step.

*Method B*—3,4,5-Trimethoxybenzonitrile (7.5 g, 39 mmoles) was dissolved in 250 ml of absolute ethanol and 10 ml of chloroform and was




*Scheme I*

[2] All melting points are uncorrected and were recorded on a Thomas–Hoover capillary apparatus. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. NMR data are delta values relative to tetramethylsilane in deuterochloroform or 3-(trimethylsilyl)propionic acid sodium salt in deuterium oxide. Starting materials and compounds not described under *Experimental* were obtained from Aldrich Chemical Co.

hydrogenated over platinum oxide (0.5 g) at 45 psi for 5 hr. The catalyst was filtered, and the filtrate was evaporated to dryness under reduced pressure to give a white powder. The hydrochloride salt thus obtained was dissolved in 30 ml of water and extracted twice with ether. The aqueous layer was made basic with 10% NaOH, saturated with sodium chloride, and extracted three times with ether. The ether extracts were combined, dried over sodium sulfate, and evaporated to dryness to give 5.6 g (75%) of pale-yellow oil with an NMR spectrum identical to that described for the product from Method A.

**3,4,5-Trihydroxybenzylamine Hydroiodide (V)**—To a magnetically stirred solution of IV (7.5 g, 38 mmoles) in 47% hydroiodic acid (75 ml, 470 mmoles) under nitrogen was added, by syringe, 43.5 ml (460 mmoles) of acetic anhydride through a septum. The reaction mixture was refluxed for 4 hr and evaporated to dryness *in vacuo*. Ethanol (100 ml) was added to the residue, and the solvent was evaporated again to yield a pale-yellow powder (5.0 g, 46%). Recrystallization from ethanol plus ether gave fine crystals, mp 196–198° dec.; NMR (deuterium oxide): 4.00 (s, 2H, $CH_2$) and 6.55 (s, 2H, aromatic).

*Anal.*—Calc. for $C_7H_9NO_3$·HI: C, 29.68; H, 3.53; N, 4.96. Found: C, 29.66; H, 3.52; N, 4.81.

**2,3,4-Trimethoxybenzylamine (VI)**—Compound VI was prepared as an oil in a 73% yield on a 5-g scale from the amide, using borohydride reduction as described for IV. It was used without further purification; NMR (deuterochloroform): 1.95 (s, 2H, $NH_2$), 3.70 (s, 2H, $CH_2$), 3.79–3.83 (m, 9H, $CH_3$), 6.50 (d, 1H, $J$ = 4.5 Hz, aromatic), and 6.85 (d, 1H, $J$ = 4.5 Hz, aromatic).

**2,3,4-Trihydroxybenzylamine Hydroiodide (VII)**—Compound VII, mp 178–181° dec., was prepared on a 7-g scale (89%) from VI by the procedure used for V; NMR (deuterium oxide): 4.15 (s, 2H, $CH_2$), 6.50 (d, 1H, $J$ = 4.5 Hz, aromatic), and 6.81 (d, 1H, $J$ = 4.5 Hz, aromatic).

*Anal.*—Calc. for $C_7H_9NO_3$·HI: C, 29.68; H, 3.53; N, 4.96. Found: C, 29.81; H, 3.70; N, 4.87.

**2,3-Dimethoxybenzylamine (X)**—2,3-Dimethoxybenzonitrile (3.29 g, 20 mmoles) was dissolved in 200 ml of absolute ethanol, and 10 ml of chloroform and 0.5 g of platinum oxide were added to this solution. The mixture was hydrogenated for 3 hr at 40 psi, the catalyst was filtered, and the solvent was evaporated. Then the residue was dissolved in a minimum amount of water and made basic with 10% NaOH. The mixture was saturated with sodium chloride and extracted three times with ether. The ether extracts were combined, dried over sodium sulfate, and evaporated to dryness to give 2.9 g (90%) of a yellow oil. The oil was used without further purification; NMR (deuterochloroform): 2.25 (s, 2H, $NH_2$), 3.8 (m, 8H, $CH_2$ and $CH_3$), and 6.81 (m, 3H, aromatic).

**2,3-Dihydroxybenzylamine Hydroiodide (XI)**—Compound XI, mp 157–158° dec., was prepared from X on a 5-g scale (60%) by the procedure used for V; NMR (deuterium oxide): 4.20 (s, 2H, $CH_2$) and 6.95 (m, 3H, aromatic).

*Anal.*—Calc. for $C_7H_9NO_2$·HI: C, 31.46; H, 3.75; N, 5.24. Found: C, 31.59; H, 3.96; N, 5.26.

**Antitumor Testing**—Antitumor activity was determined as percent T/C values, with T/C ≥ 125% defined as statistically significant (Table I). Dose–response studies were carried out for each compound according to published National Cancer Institute protocols (10). Six mice per dose were inoculated intraperitoneally with $10^6$ P-388 leukemia cells on Day 0. Control (untreated) mice usually died on about Day 11. Mice receiving drug were treated on Days 1–9 with intraperitoneal doses of the compound under investigation. Physiological saline (0.9%) was the vehicle. The mice (average weight 20 g) were weighed on Day 5; the weight difference between treated and control mice (T − C) was an indication of dose toxicity. Weight losses greater than 4 g were considered excessive.

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# Aluminum Chlorohydrate III: Conversion to Aluminum Hydroxide


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Received October 3, 1980, from the **Department of Industrial and Physical Pharmacy and the* ‡*Department of Agronomy, Purdue University, West Lafayette, IN 47907.* Accepted for publication December 8, 1980.



**Abstract** □ Bayerite, an aluminum hydroxide polymorph, readily forms when the hydroxyl to aluminum ratio of aluminum chlorohydrate is raised to 3 by titration with sodium hydroxide. Dilution of aluminum chlorohydrate solutions with water leads to the formation of gibbsite, another aluminum hydroxide polymorph. The mechanism of conversion in each instance is related to the structure of the $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ complex.



**Keyphrases** □ Aluminum chlorohydrate—conversion to aluminum hydroxide □ Aluminum hydroxide—conversion from aluminum chlorohydrate □ Antiperspirant activity—conversion of aluminum chlorohydrate to aluminum hydroxide


Aluminum chlorohydrate recently was shown (1) to be the $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ (I) complex described by Johansson *et al.* (2). This structure is unusual in that it is composed of a central aluminum atom in tetrahedral configuration surrounded by 12 octahedral aluminum atoms. The charge is neutralized by chloride ions. In contrast, aluminum hydroxide is composed exclusively of aluminum in octahedral configuration. The basic unit of aluminum hydroxide is a six-member ring composed of aluminum in octahedral configuration formed by a dehydration–deprotonation reaction (3). The relationship between aluminum chlorohydrate and aluminum hydroxide was demonstrated by examining the effect of neutralizing aluminum chlorohydrate by the addition of sodium hydroxide and the effect of diluting with water.

## EXPERIMENTAL

Aluminum chlorohydrate was obtained commercially as a 50% (w/w) solution[1]. The effect of increasing the hydroxyl to aluminum ratio of aluminum chlorohydrate to 3 was studied by adding 500 ml of 0.394 *N* NaOH at a rate[2] of 10 ml/min to 500 ml of an aluminum chlorohydrate

[1] Lot 8473, Wicken Products, Huguenot, N.Y.

[2] Buchler Polystaltic Pump, Buchler Co., Fort Lee, N.J.




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