Radiohalogen-Labeled Imaging Agents. 3. Compounds for Measurement of Brain Blood Flow by Emission Tomography

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The radioiodine-labeled amines currently available as brain-imaging agents, based on our previous work and that of others, are prepared either by exchange labeling or by direct iodination of a protected intermediate. The intrinsic slowness of these processes limits their potential for use with the positron-emitting $^{122}$I as it has a half-life of only 3.6 min. This isotope has advantages of a low dose to the patient and availability from a generator containing the parent $^{122}$I $^{122}$Xe. To develop a radiopharmaceutical in which $^{122}$I could be utilized, we prepared a number of secondary and tertiary amines (maintaining the 2,5-dimethoxy substitution pattern which allows direct iodination at the 4-position) with $^{122}$I. The organ distributions of these compounds were studied, and the best properties were found in the $N,N$-dimethyl homologue (2,5-dimethoxy-$N,N$-dimethyl-4-iodoamphetamine). This compound was successfully synthesized in a matter of seconds, with a chemical yield and radioactive purity both in excess of 90% and an incorporation efficiency of radioiodine of around 40%.

Paper 1 of this series described an amphetamine analogue, 2-(4-$^{122}$Iiodo-2,5-dimethoxyphenyl)isopropylamine, which was the first reported radiohalogen-labeled organic compound that showed uptake and reasonably long retention in normal human brain. The second paper described the synthesis of the analogous radiiodinated agent, 2-(4-$^{122}$Iodo-2,5-dimethoxyphenyl)isopropylamine (1p), which showed first-pass extraction in the monkey from blood to brain. Because of this property, we proposed...
that it would be useful as a brain-imaging agent in nuclear medicine.3 Additional analogues of this compound were subsequently reported by Winchell et al.:5 one of them, N-isopropyl-2-(p-iodophenyl)isopropylamine (termed IMP), was chosen for clinical trials on the basis of its high brain/blood ratio.

Clinical trials with 131I-labeled IMP using single photon emission computed tomography have shown the usefulness of this agent in measurement of regional brain blood flow.6,7 Perfusion deficits in brain, many not detectable by CAT scanning, were shown in patients with stroke. Hyperperfusion was seen in patients with epileptic foci. Other more distantly related compounds labeled with radiiodine have also been reported as potential brain imaging agents. 5,8

All of the above agents, with the exception of our original compound with the 2,5-dimethoxy ring substitution pattern, are made by first synthesizing the halogenated compound and then labeling by exchange. Such a process, while convenient for manufacture, has two limitations: (1) the specific activity is inherently limited because of the presence of unlabeled compound, and (2) the exchange labeling reaction is relatively slow; this limits the usable isotopes to those of fairly long half-life. Thirteen-hour 131I is the most useful isotope for this application because its $\gamma$ energy is ideal for single photon imaging devices.

Positron emission tomograph (PET) imaging systems construct tomographic images by utilizing detection of the coincident 0.51-MeV $\gamma$ rays from decay of positrons. PET images contain quantitative data on the regional concentration of the isotope within the human body without interference by overlying tissue. PET devices require positron-emitting radioisotopes, and the most feasible among the halogens that are suitable in terms of half-life and radiation dose to the patient are 1.7-h 76Br and 3.6-min 123I. Of these, 123I has two advantages; it has a very short half-life, making possible repeat studies at short intervals with lower total radiation dose, and it is the daughter of 122Xe. A generator system has been described by which the 122I daughter can be repeatedly extracted carrier-free from the 122Xe parent.9 This would allow the production and shipping of 123I generators to institutions that do not have cyclotrons.

It is with these considerations in mind that we have investigated the series of compounds reported here. The 2,5-dimethoxy substituents sterically direct halogenation at the para position; if the primary amine function of the precursor is protected, oxidative side reactions are avoided.10 In our first studies this protection was afforded by a phthalide group, which was removed after iodination. In order to provide the most rapid possible synthesis with 122I, we decided to investigate 2-(4-iodo-2,5-dimethoxyphenyl)isopropylamine with various nitrogen substitutents to determine the stability of the precursor to direct iodi- nation. If high brain uptake could be found with one or more of these species, the removal of the protective groups would be unnecessary, and sufficient rapidity of synthesis might be available to utilize labeling with 123I, as well as with more conventional isotopes, such as 131I.

Chemistry. To evaluate a large number of $N$-substitution patterns for organ distribution, we developed a procedure that allowed a single radiolabeled compound to serve as a precursor (Scheme I). The product of the reaction between $p$-dimethoxybenzene and iodine monochloride depended upon the solvent employed. The relatively polar acetic acid yielded exclusively the diiodo derivative 2a, whereas the use of methylene chloride provided largely the chloro-iodo counterpart 2b. Trials with nonpolar solvents, such as hexane, led only to monohalogenation. Reaction of 2 with butyllithium, followed by treatment with $N$-methylformanilide, led to the replacement of the iodine atom with a carboxaldehyde function. In the reaction of 2a to 3a, a small amount of dilithiation yielded, as an impurity in 3a, the known 2,5-dimethoxyterephthalaldehyde 3c. The ammonia-catalyzed reaction of 3a with nitroethane and the reduction of the resulting nitrostyrone 4 with iron provided the ketone 5a with no complications. A number of environments were explored for the exchange of iodine for radioiodine in 5.
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Scheme II

\[
\begin{align*}
\text{6} & \quad \text{CH}_3O\text{C}\text{H}_2\text{O}^- \\
& \quad \text{CH}_2\text{NH}_2\text{Cl} \\
\text{7} & \quad \text{CH}_3\text{O}\text{C}\text{H}_2\text{O}^- \\
& \quad \text{N(CH}_3)_2\text{I} \\
\text{1m} & \quad \text{CH}_3\text{O}\text{C}\text{H}_2\text{O}^- \\
& \quad \text{N(CH}_3)_2\text{I}_3\text{ICl} \\
& \quad \text{131}^\text{I} \\
\end{align*}
\]

(Table II); the most satisfactory was the use of Na\textsuperscript{131}I in acetic acid at 140 °C. Reductive amination of 6b with various primary or secondary amines and sodium cyanoborohydride provided the corresponding product 1.

The dimethoxy derivative having the highest brain uptake (1m) was prepared by the reductive amination of 2,5-dimethoxyphenylacetone 6 with dimethylamine (Scheme II), followed by the direct iodination of the resulting N,N-dimethyl-2-phenylisopropylamine 7 to 1m with \textsuperscript{131}ICl. A nonradioactive reference sample of 1m was prepared by the direct dimethylation of 1r. Several iodination procedures were evaluated from the viewpoint of speed, radiochemical purity, radioincorporation yield, reaction scale, and ease and speed of separation from by-products.

Results and Discussion

The uptake in each of the measured organs of each of the \textsuperscript{131}I-labeled compounds is shown in Table I, as percent of the injected dose in the total organ and as percent of injected dose per gram in each organ. Desirable features of a brain blood flow agent are that it be taken up in brain sufficiently to obtain γ ray imaging and that it be removed from blood rapidly so that the radioactivity imaged in brain will not have a significant contribution from circulating blood. Thus, the parameters of interest are the percent of the injected dose appearing in the brain or other organs and the ratio of organ to blood radioactivity normalized on a weight basis. The time course of these parameters is also of interest because, when a very short-lived isotope is used, it is desirable that optimal values be reached within a few half-lives. Hence, data were obtained at 5 and 30 min for each compound.

Compound 1m had the highest percent uptake in brain, 3.4%, and also the highest value of brain to blood ratio, 9.0. For compound 1n, this ratio was 5.0 and for the compound we first reported,\textsuperscript{3} 1r, it was 4.7. Thus, the best candidate for a useful brain blood-flow imaging agent is 1m, the N,N-dimethyl analogue. Direct iodination of the precursor 7 to produce 1m (see Experimental Section) has produced chemical yields of 90% in 10 s, a yield and speed satisfactory for use with \textsuperscript{125}I. A useful dose of 12 mCi of \textsuperscript{125}I-labeled 1m will remain 14.4 min (4 half-lives) after extraction from a 200 mCi \textsuperscript{125}Xe generator. Methods are currently under development for purification of 1m within this time span. The reaction yields reported here were achieved at a 1 µg level, obviating pharmacological problems in human applications.

\textsuperscript{131}I-labeled 1m was further studied by injecting 75 µCi into a dog and measuring brain uptake and blood radioactivity as a function of time, with results shown in Figure 1. Activity in the brain reached a maximum at 5 min, and decreased to 65% of its maximum value by 30 min. Blood activity fell rapidly and reached an almost constant value by 5 min, with a brain/blood ratio of approximately 9, in good agreement with the value found in rats. The enlarged image of the head showed clearly the concentration in brain. It should be noted that the gamma energy of \textsuperscript{131}I and the scanner used produce images of relatively poor resolution. The uptake values for brain are not corrected for tissue attenuation and isotope in overlying tissue; the values on the ordinate for blood and brain were calculated on a slightly different basis (see Experimental Section). The \textsuperscript{131}I provided the convenience of long half-life, and the entire animal was easily scanned with the Mark II scanner, but \textsuperscript{125}I with 160-keV γ rays and a scintillation camera would produce much better images. Thus, 1m appears to have excellent characteristics for the type of brain blood-flow agent we are seeking: rapid uptake and relatively long retention in brain, rapid clearance from blood, a high brain/blood ratio, and rapid synthesis by direct iodination. Studies are now underway to develop the requisite technology and chemistry for production of the \textsuperscript{131}I-labeled 1m from a \textsuperscript{125}Xe-\textsuperscript{125}I generator.

Further study of Table I reveals other compounds of possible interest as radiopharmaceuticals. \textsuperscript{99m}Tc micro-particle agents for scanning of lung are important for diagnosis of emboli and perfusion deficits in a variety of illnesses, and ventilation imaging is currently done with isotopes of gases. Examination of the lung section of Table I reveals five compounds (1a–d, f) with a combination of high percent uptake, high percent uptake per gram, and high organ/blood ratio. All of these compounds would appear to be of interest for further evaluation as lung imaging agents. We have earlier reported lung uptake with the bromine congener\textsuperscript{1} and suggested use of the iodine analogue as a lung imaging agent.\textsuperscript{3} In a recent report,\textsuperscript{4} the radiation dose to various organs was examined based on uptake of \textsuperscript{131}IMP, another amphetamine analogue.


Particular emphasis was placed on the high uptake of the 125I in the retina and its relatively long retention there, a phenomenon we first reported for 1r in 1978. The high radiation dose to the retina results from the long biological retention; the use of 122I to label amine its retina uptake in future studies. Although it has a high lung uptake before it can deliver an appreciable dose to the retina. Because of the possibility that 1m could also be used for a 131I-labeled radiopharmaceutical, however, we will examine its retina uptake in future studies.

Radiopharmaceuticals for imaging the heart are also of interest for study of cardiac disorders. Because the heart is essentially surrounded by lung in the human, it is important that uptake of the agent by the lung be as low as possible. Compound II has a significant heart uptake, and although it has a high lung uptake as well, this compound or analogues of it may warrant further investigation for application as a cardiac imaging agent.

Experimental Section

Melting points were taken on a Laboratory Devices Mel-Temp apparatus and are uncorrected. Infrared spectra were obtained on a Beckman Acculab spectrometer. NMR spectra were obtained on a Nicolet FT-200. Distillations were carried out bulb to bulb in an Aldrich Kugelrohr oven. Microanalyses were performed with a Beckman Acculab spectrometer. Injections were made in the tail veins of rats restrained in plastic holders and lightly anesthetized with ether. Bolus injections of 0.2 mL of a saline solution containing 10–20 μCi of the 131I-labeled compound were made, and the rats were sacrificed by decapitation. The organs of interest were excised immediately after sacrifice, weighed, and counted in a Searle Model 1185 gamma well counter. A calibrated external standard was prepared by serial dilution of the injected compound, and percent dose per organ of the injected amount was determined.

Blood clearance in the beagle dog was measured by serial venous sampling. Brain uptake was measured by whole-body scanning with the Anger Mark II whole-body scanner and determining radioactivity in the brain from a region of interest defined over the brain with an HP5040A data analyzer. The percent dose per organ was calculated from the events in the region of interest divided by the events in a region covering the whole body times 100, divided by an assumed brain weight of 90 g.

2,5-Diiodo-1,4-dimethoxybenzene (2a). To a solution of 6.9 g of p-dimethoxybenzene (50 mmol) in 50 mL of acetic acid there was added over the course of 5 min a solution of 20 g of ICl in 20 mL of acetic acid. The mixture was heated on a steam bath for 2 h and then cooled with external ice-water, resulting in the formation of a heavy steel-gray crystalline mass. This was removed by filtration, washed sparingly with cold acetic acid, and suspended in 200 mL of water. With good stirring, there was added small portions of Na2S2O4 until the color of the suspended solids had changed from gray to white. This product was removed by filtration, washed with water, and when reasonably dry, recrystallized from 50 mL of boiling acetonitrile. There was thus obtained 7.1 g (58% yield) of a white product, mp 161–165°C. An analytical sample that was recrystallized again from acetonitrile had mp 167–168°C (lit.11 mp 171°C). NMR (CDCl3) δ 3.82 (6 H, OCH3), 7.19 (2 H, Ar H). Anal. (C8H10I2O) C, H.

2-Chloro-5,5-diiodo-1,4-dimethoxybenzene (2b). To a solution of 6.9 g of p-dimethoxybenzene (50 mmol) in 30 mL of CH2Cl2 there was added 20 g of I2 in 30 mL of CH2Cl2. After stirring for 1 h, the solution was held at reflux for 20 min (steam bath), cooled, and diluted with water. Solid Na2S2O4 was added with
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CHSCN, filtered free of solvent, and recrystallized from 35 mL of pale-yellow crystals of methanol, filtered free of solvent, air-dried (7.5 g), and finally a pale-yellow solid. This product was triturated under 20 mL of white precipitate to a thick suspension. This was stirred for 10 min, then heated at reflux for about 10 min, and then added to 800 mL of water acidified with HCl. Yellow solids were removed by filtration and washed with ether and acetone (see below). The resulting clear mother liquors were separated, and the organic fraction was stripped of volatiles to yield, after air-drying, 17 g of a pale-yellow solid. This product was triturated under 20 mL of methanol, filtered free of solvent, air-dried (7.6 g), and finally recrystallized from 60 mL of boiling 95% ethanol to yield 5.2 g of pale-yellow crystals of 3a: mp 156–157°C; NMR (CDCl₃) δ 3.88 (3 H, OCH₃), 3.90 (6 H, OCH₃), 7.22 (1 H, ArH), 7.47 (1 H, ArH), 10.40 (1 H, CHO); IR (Μ.Ο. mull 1675 cm⁻¹), 1105, 1029, 969, 878, 717, 663 cm⁻¹. Anal. (C₇H₇O₃Cl) C, H.

4-Iodo-2,5-dimethoxybenzaldehyde (3a). To a stirred suspension of 19.6 g (50 mmol) of N-butyllithium in hexane (cooled externally with ice-water) there was added 34 mL of 1.6 M 2-propanol (mp 114–115°C) and then from acetonitrile (mp 115–116°C). NMR (CDCl₃) δ 3.84 (6 H, OCH₃), 6.86 (1 H, ArH), 10.40 (1 H, CHO); IR (Μ.Ο. mull 1675 cm⁻¹), 1105, 1029, 969, 878, 717, 663 cm⁻¹. Anal. (C₇H₇O₃Cl) C, H.

Attempts to iodinate 2,5-dimethoxybenzaldehyde in the 4-position with IC₁ employing the procedure in ref 13 were unsuccessful.

4-Chloro-2,5-dimethoxybenzaldehyde (3b). In a manner similar to the preparation of 3a above, 2b was converted to 3b, again with the concomitant preparation of a small amount of 3c as an impurity. The product was pale yellow, obtained in a yield of 49%, and had a mp 93–95°C after recrystallization from methanol: NMR (CDCl₃) δ 3.89 (6 H, OCH₃), 7.07 (1 H, ArH), 7.26 (1 H, CHO); IR (Μ.Ο. mull 1675 cm⁻¹), 1060, 1031, 983, 880, 730 cm⁻¹. Anal. (C₇H₇ClO₃) C, H.

1-(4-Iodo-2,5-dimethoxyphenyl)-2-nitropropene (4). To a solution of 4.8 g of 3a (16.4 mmol) in 100 mL of nitroethene was added 0.3 g of ammonium acetate, and the mixture was heated on a steam bath for 8 h. The orange-red solution was decanted from a small amount of insolubles and stripped of nitroethane in vacuo, and the residue was treated with 20 mL of boiling methanol. Crystals of 4 formed spontaneously, removed by filtration, and recrystallized from 65 mL of boiling methanol to yield 2.3 g of product: gold crystals with mp 117–118°C; yield 40%. An analytical sample obtained by recrystallization from methanol melted at 119–120°C. Anal. (C₁₅H₁₂NO₃) C, H, N.

1-(4-Iodo-2,5-dimethoxyphenyl)-2-propanone (5a). A solution of 2.1 g of 4 (6.6 mmol) in 10 mL of hot acetic acid was added to a suspension of 4 g of electrolytic iron in 20 mL of warm acetic acid. Upon heating on a steam bath, the reaction mixture became very dark and finally there was the deposition of white solids beneath a heavy black oil. Heating was continued for 1.2 h, the reaction mixture was poured into 500 mL of water, a small amount of unreacted iron and an insoluble brown residue was removed by filtration and washed with CH₂Cl₂, and the mother liquors were separated, with the aqueous phase being extracted with an additional 2× 75 mL of CH₂Cl₂. The organic phases were pooled, the solvent was removed in vacuo, and the residue (3.9 g of a pale amber oil) was distilled at 0.4 mmHg to yield a fraction, bp 120–130°C, of a colorless oil (1.1 g, yield 52%) that spontaneously crystallized. Recrystallization of an analytical sample from methylecyclopentane gave white crystals mp 62–65°C; NMR...
mg of the amine R'R''NH hydrochloride in 0.2 mL of methanol, and approximately 50 mg of NaCNBH₃. The slurry was shaken occasionally over a period of 3 days. The reaction product was partitioned between CH₂Cl₂ and dilute sulfuric acid, the aqueous phase was made basic with dilute sodium hydroxide and reextracted with CH₂Cl₂, and the solvent was removed with a stream of warm dry nitrogen. The radioyield was determined by direct counting of the reaction product before and after purification, and the radioactivity was determined by TLC assay of the purified base fraction on silica gel, employing the solvent system ethyl acetate/methanol/ammonium hydroxide in a 54:4:1, v/v ratio. These results are tabulated in Table III.

N,N-Dimethyl-2-(2,5-dimethoxyphenyl)isopropylamine (1m, Nonradioactive Form, from 1l). A solution of 0.4 g of 1l as the hydrochloride salt (see ref 2) in 12 mL of methanol and 4 mL of 40% CH₃OH was stirred magnetically and treated with 1 g of NaCNBH₃. Concentrated HCl was added periodically to maintain pH 5-6. After 4 h, the reaction mixture was poured into 250 mL of water, made strongly basic, and extracted with 3 × 75 mL of CH₂Cl₂. The extracts were pooled and extracted with 2 × 75 mL of dilute H₂SO₄, and the extract, after pooling and being made basic (4 N NaOH), was again extracted with CH₂Cl₂. Removal of the solvent yielded 0.38 g of a colorless oil, which was dissolved in 2 mL of 2-propanol and treated with a warm solution of 0.13 g of oxalic acid dihydrate in 10 mL of methanol, followed by 150 mL of a stream of NH₃ gas. The reaction product was stirred at room temperature, and concentrated HCl was added as needed to maintain the pH at about 6. The crude exchange product from this process averaged 74% temp., radioyield, %

<table>
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<th>exchange media</th>
<th>temp, °C</th>
<th>radioyield, %</th>
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</table>

*See Scheme I for structure. *Not determined. Prepared by iodination of the preformed amine (see Experimental Section).

The reductive aminations employing dimethylamine (to yield 1m), diethylamine (to yield 1n), and β-methoxyethylamine (to yield the corresponding N-2-methoxyethyl) analogue of 1l) all displayed multiple spots.
and a void volume at 1.42 min. With the above concentrations, the radiopurity and chemical purity were consistently in excess of 90%, and the radioincorporation efficiency was in the 30 to 40% range. The reaction gave similar results at 10 and 100 times these concentrations, but at one-tenth this concentration the yields were drastically reduced.

Iodination of 7 to form 1m and the direct synthesis of 1m, when performed with 125I in acetic acid but in the absence of perchloric acid, resulted in a maximum radioincorporation of 12%.

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**Registry No.** 1a, 9006-55-2; 1b, 9006-54-3; 1c, 9006-55-4; 1d, 9006-56-5; 1e, 9006-57-6; 1f, 9006-58-7; 1g, 9006-59-8; 1h, 9006-60-1; 1i, 9006-61-2; 1k, 9006-67-8; 1l, 9006-62-3; 1m (unlabeled), 85563-10-8; 1m-HCl (unlabeled), 9006-51-0; 1m (unlabeled), 85563-10-8; 1n, 9006-63-4; 1o, 9006-64-5; 1p, 9008-18-4; 1q, 9006-65-6; 1r-HCl (unlabeled), 42203-78-1; 1r, 657760-98-2; 2a, 51860-21-5; 2b, 9006-46-3; 3a, 9006-47-4; 3b, 9006-48-5; 3c, 7310-97-8; 4a, 9006-44-1; 5a, 9006-49-6; 5b, 9006-50-9; 6, 14283-24-4; 7, 87707-78-2; 7-oxa, 9006-45-5; 7-dimethoxybenzene, 150-78-7; N-methylformanilide, 83-61-8; methanimine hydrochloride, 593-51-1; isopropylamine hydrochloride, 15572-56-2; cyclopropenemethanemethanimine hydrochloride, 7252-53-1; hexanimine hydrochloride, 142-81-4; dodecanemethanimine hydrochloride, 929-73-7; benzenenemethanimine hydrochloride, 3287-99-6; hydrazine hydrochloride, 1401-17-1; hydroxylamine hydrochloride, 2540-11-1; aminocetanitrite hydrochloride, 6611-14-9; 2-thioethanemethanimine hydrochloride, 156-57-0; 2-methoxethanemethanimine hydrochloride, 18600-40-3; 3-N,N-dimethyl-1,3-propanediamine hydrochloride, 7742-45-6; diethylamine hydrochloride, 688-65-4; N-methyl-2-propanemethanimine hydrochloride, 54568-61-6; N-methylbenzenemethanimine hydrochloride, 42870-70-2; N-methylbenzenemethanimine hydrochloride, 13426-94-3; dimethylaniline hydrochloride, 508-59-2.

**Antihypertensives.** *N-1H-Pyrrol-1-yl-3-pyridazinamines*

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The pathogenesis of hydralazine-induced lupus erythematosus has been correlated with its rate of hepatic acetylation.1 With the discovery of a novel urinary hydralazine metabolite in man, namely, 3-(hydroxymethyl)-s-triazolo[3,4-a]phthalazine, the hypothesis was advanced2 that the functional alcoholic group might provide a handle for the formation of a covalent bond to a protein and, thus, to production of antibodies to the metabolite. The recently discovered mutagenic activity of hydrazine derivatives of general formula VI are hydrazino derivatives of general formula VITable III(Scheme I), where R represents a tertiary amino group. References3,4 for those compounds already known are in Table IV. The new analogues were prepared starting from 3,6-dichloropyridazines I, which were reacted with secondary amines in the presence (method A) or absence (method B) of a solvent, to give 3-amino-6-chloropyridazines II (Table I). The substitution of the chlorobenzyl atom with hydrazine to give V was achieved by one of the following three procedures. In the first procedure, reaction with hydrazine hydrate as a solvent (method F, R1 = H) was found to be useful only when the final product had a relatively low water solubility. In most cases, the isolation of V as the hydrochloride involved troublesome crystallizations in order to satisfactorily eliminate hydrazine hydrochloride, and the final yields were generally very low. In some cases, after elimination of the hydrazine hydrate, the residues containing the compounds V were used as such for the synthesis of VI. In the second procedure, compounds V were isolated as the benzaldehydrazones III (Table II), which were easily hydrolyzed in dilute mineral acids when concomitant steam distillation of the benzaldehyde was carried out (methods C and E). In the third procedure, the hydrochlorides of II were reacted with tert-butyl carbazate in methylcellosolve, and after mild hydrolysis of the resulting compound IV, the correct hydrochloride, the residues containing the compounds V were isolated as the hydrazones III (method D). One compound, V-24, with a methyl on the hydrazino group (R1 = CH3) was prepared by methylation of the corresponding acetaldehyde hydrazone III-15, followed by hydrolysis of the resulting compound IV. The correct position of the R1 methyl group was demonstrated by catalytic reduction to the aminopyridazine XI. The new intermediates V are reported in Table III.


