of enzyme used in the individual assays was established by measuring the "burst" obtained at 410 nm with NPGB.[9] Following this, the ability of a new nitrophenyl ester to tie up the enzyme in an acyl enzyme form could be assessed, in a separate mixture containing the same aliquot of enzyme, by a subsequent addition of NPGB to the mixture of the ester and enzyme. A diminution of the burst indicated net acyl enzyme formation produced by the ester under test.

The following procedure was found to give the most reproducible results. Two-ml cells containing 1.98 ml of buffer (reference cell) or buffer plus enzyme (sample cell) were balanced. With the recorder turned off, the inhibitor to be tested was added to the reference cell with mixing and then to the sample cell, mixing being completed within 10 sec, after which time the recorder was turned on. If nitrophenol was released, time was allowed to elapse until 1–2 molar equiv of color was produced. The free enzyme concentration was then determined. For this purpose, 20-μl pipettes loaded with stock NPGB were prepared. Pipettes with a constriction retained their load of reagent while in a horizontal position, permitting rapid manipulation. The recorder was turned off, and NPGB was added to the sample cell first and then to the reference cell, with inversion of both for mixing. The maneuver could be completed within 30 sec at which time the recorder was turned on. The observed burst was corrected for the change occurring during the elapsed time by extrapolation of the post-burst rate back 30 sec (as shown in Figure 1).

In some cases, due to the low solubility of the reagent, 100 μl or more of DMF was carefully layered over the enzyme solution. The reagent was added to this layer prior to mixing. DMF in the amounts used (under 10% v/v) did not affect the stability of the enzymes nor the extent of acyl enzyme formation although it appeared to slow the acylation rate somewhat.

Inhibition of Coagulation. A reaction mixture composed of concentrated thrombin solution (20 μl), the ester to be tested (0.10 ml of a 10^{-3} *M* solution in DMF), and 1.70 ml of 0.05 *M* imidazole buffer, pH 7.38, 0.15 *M* in sodium chloride was prepared. The final thrombin concentration was 2.4×10^{-6} *M*. Aliquots removed for coagulation assay ranged from 20 to 75 μl (as required to maintain a clotting time of 13–19 sec in the Kline assay[21]) and were added to 0.0139 *M* imidazole buffer, pH 7.38, 0.15 *M* in sodium chloride to achieve a final volume of 0.5 ml for assay.

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Absolute Configuration of α-Methyldopamine Formed Metabolically from α-Methyldopa in Man†

Kent S. Marshall and Neal Castagnoli, Jr.*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94122.
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The absolute configuration of metabolic α-methyldopamine [1-(3,4-dihydroxyphenyl)-2-aminopropane, **2a**] has been unambiguously established. Urine obtained from a hypertensive patient on α-methyldopa [(*S*)-(−)-3-(3,4-dihydroxyphenyl)-2-methylalanine, **1**] therapy was acidified, lyophylized, and extracted with methanol. The methanol extracts were methylated with diazomethane and subsequently purified by acid–base partitioning. The basic fraction, which was shown to contain 1-(3,4-dimethoxyphenyl)-2-aminopropane (**7**), was derivatized with the chiral reagent (*S*)-(−)-*N*-pentafluorobenzoylprolyl 1-imidazolide (**6**). The resulting amide was shown to have a glpc retention time corresponding exactly to the amide formed from authentic (*S*)-(+)-amine **7a**.

The mode of action and metabolic fate of the antihypertensive agent α-methyldopa [(*S*)-(−)-3-(3,4-dihydroxyphenyl)-2-methylalanine, **1**] have been studied by numerous groups.[1] Since it is generally held that the pharmacologic effects of this compound are dependent on its conversion to active metabolites, a more detailed description of the relative stereochemistries of the metabolites should be of value in understanding the mode of action of the drug. As part of our studies on the metabolic fate of α-methyldopa, we have determined and report here the absolute configuration of α-methyldopamine [1-(3,4-dihydroxyphenyl)-2-aminopropane, **2a**] formed *in vivo* by a patient on α-methyldopa therapy.

Sjoerdsma and Udenfriend[2] have shown that the pharmacological activity of **1** is found only in the levorotatory enantiomer, the absolute configuration of which has been independently established by Terashima, *et al.*,[3] and Tristram, *et al.*,[4] to be *S*.

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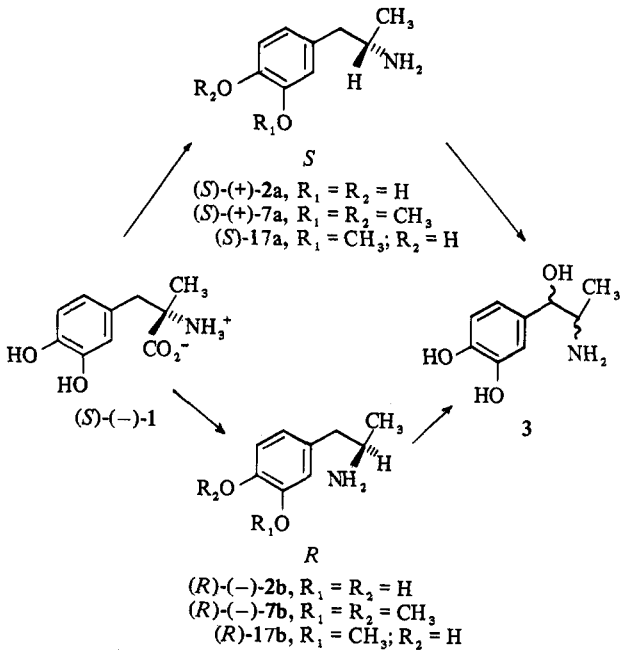
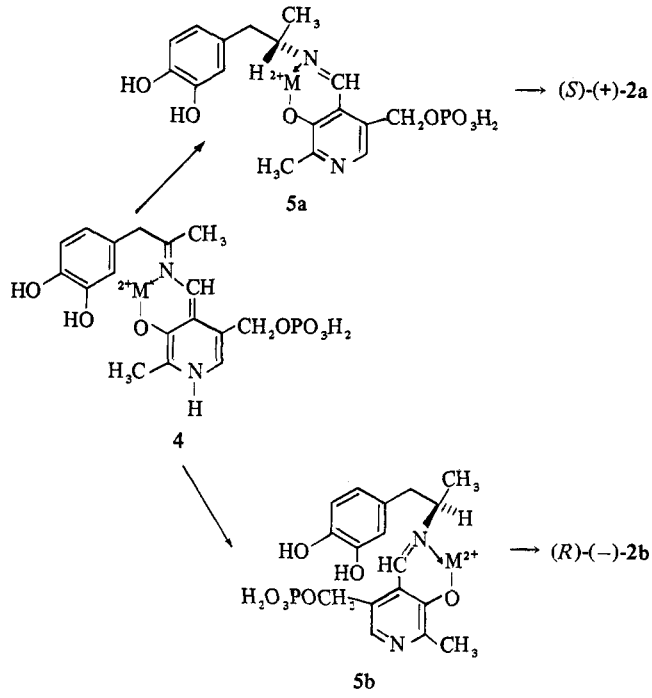


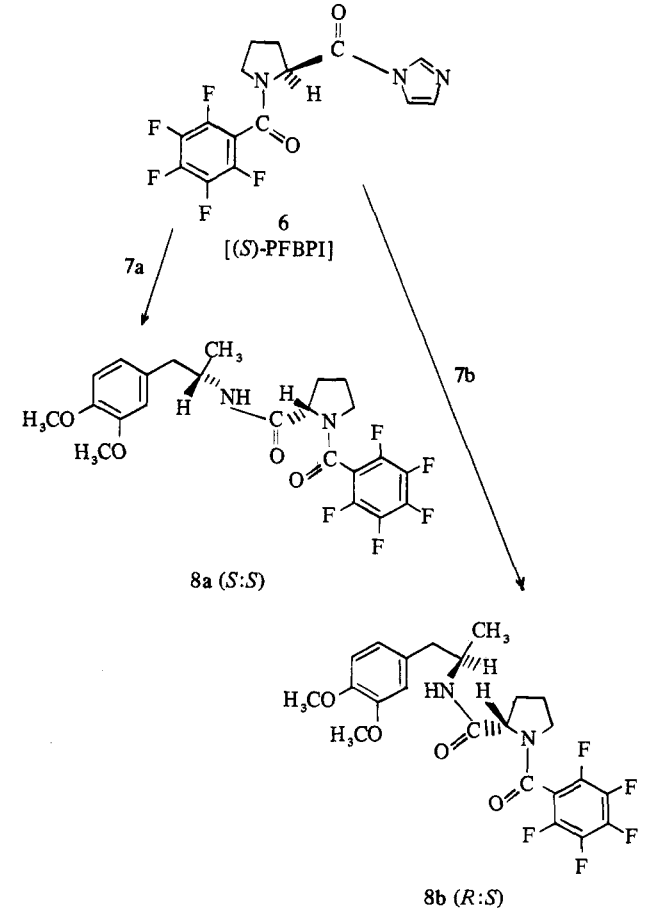
One of the major metabolic pathways of **1** involves its decarboxylation to α-methyldopamine (**2**) which subsequently undergoes β-hydroxylation to yield α-methylnorepinephrine [1-(3,4-dihydroxyphenyl)-1-hydroxy-2-aminopropane, **3**]. The decarboxylation of **1** in mammalian tissues is catalyzed by aromatic L-amino acid decarboxylase,[5] a pyridoxal 5-phosphate, and metal requiring enzyme.[6] The decarboxylation proceeds *via* an original Schiff base adduct to generate **4**, a species which has lost the stereochemical integrity possessed by α-methyldopa. Subsequent protonation and hydrolysis of **4** can proceed stereospecifically to yield either optically active amine **2a** or **2b**, stereoselectively to yield a predominance of one enantiomer, or with complete lack of stereocontrol to yield racemic **2**. The protonation step (corresponding to **4** → **5a** or **5b**) during the *in vitro* (carried out in D_2O) decarboxylation of (*S*)-tyrosine has been reported by Mandeles, Koppelman, and Hanke[7] to be stereospecific. Further, *in vitro* decarboxylation studies in D_2O by Belleau and Burba[8] indicate the overall conversion of (*S*)-tyrosine to (*R*)-1-*d*-tyramine proceeds with retention of configuration.

A knowledge of the degree of stereoselectivity of the decarboxylation becomes very important in understanding α-methyldopa's mode of action, since some pharmacologic evidence[1a] suggests that the observed biological activity of the drug may be dependent on this conversion. This information is also important when considering the conversion of α-methyldopamine into α-methylnorepinephrine where a second asymmetric carbon atom is introduced.

Waldeck[1h] has suggested that metabolic α-methyldopamine has the *S* configuration. This assignment was based on the configuration of α-methylnorepinephrine (formed *in vivo* from α-methyldopa) which has been tentatively assigned[9,10] as 1*R*:2*S*. However, α-methylnorepinephrine's configuration has not, as of yet, been unambiguously determined. To date, an unambiguous absolute configuration determination for metabolic α-methyldopamine has not been reported.

Matin, *et al.*,[11] have developed a new method for predicting absolute configurations of 1-aryl-2-aminopropanes using the chiral derivatizing reagent (*S*)-(−)-*N*-pentafluorobenzoylprolyl 1-imidazolide (PFBPI, **6**) and gas-liquid partition chromatography (glpc). The PFBPI technique for investigations of configuration involves the formation of the amide derivatives **8a** and **8b** from the combination of **6** with the bis-*O*-methyl ethers of **2** (*i.e.*, **7a** and **7b**). These amides are diastereomers and, therefore, may have different glpc retention times.





The determination of the glpc retention times of amides **8a** and **8b** can be carried out using very minute quantities of amines and chiral reagent. The glpc sample is taken directly from a benzene reaction mixture of amine and PFBPI. In order to use the PFBPI system, the α-methyldo-

pamine excreted in the urine of patients on α-methyldopa therapy must be isolated in at least crude form by methods which will not affect its asymmetric center. The standard ion exchange and paper chromatographic separation and purification techniques have been used[1c,e-g] with reasonable success but without regard to possible stereochemical alterations. Pratesi and coworkers[12] were able to O-methylate both synthetic enantiomeric α-methyldopamines **2a** and **2b** using excess diazomethane without affecting the stereochemistry of the isopropylamine side chain. Coupling this with the Buhs and coworkers'[1c] observation that α-methyldopamine is excreted at least in part as the free base provided us with the key to the isolation problem.

The absolute configurations of both (+)- and (−)-1-(3,4-dimethoxyphenyl)-2-aminopropane (**7a** and **7b**) have been well established[12-14] as *S*-(+) and *R*-(−), respectively. The preparation and detection of the diastereomeric amides **8a** and **8b** were performed using authentic samples of dimethoxyamines (*S*)-(+)-**7a** and (*R*)-(−)-**7b**, respectively. The (*R*)-(−)-amine **7b** gave the corresponding amide **8b** with a peak retention time of 28.5 min. The enantiomeric (*S*)-(+)-amine **7a** yielded an amide **8a** with a peak retention time of 33.4 min. Both authentic samples contained very small amounts of the other enantiomer.

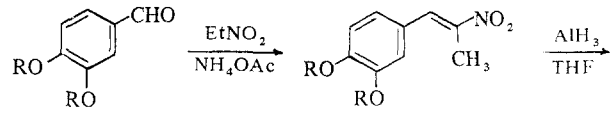
In order to show that the PFBPI reagent does not react preferentially with one of the enantiomeric amines and that the dimethoxyamines could be prepared and isolated in crude form from urine, it was necessary to prepare both racemic 1-(3,4-dimethoxyphenyl)-2-aminopropane (**11**)[14,15] and α-methyldopamine (**2**). Aluminum hydride reduction[16] of 1-(3,4-dimethoxyphenyl)-2-nitropropene (**10**)[15b,17] prepared by the base-catalyzed condensation of nitroethane with 3,4-dimethoxybenzaldehyde (**9**)‡ gave **11** in excellent yield as a colorless oil which crystallized on standing. Derivatization of **11** with PFBPI followed by glpc analysis gave a chromatograph showing two equally intense peaks with retention times of 28.5 and 33.4 min corresponding exactly with those observed for individual diastereomeric amides **8a** and **8b**. Therefore, the chiral reagent **6** shows no stereochemical preference during amide formation.

The preparation of (±)-α-methyldopamine (**2**) was accomplished using the same basic approach, however, with 3,4-dibenzyloxybenzaldehyde (**12**)[18],‡ as the starting material (hydrobromic acid hydrolysis of **11** would also yield **2**). Aluminum hydride reduction of 1-(3,4-dibenzyloxyphenyl)-2-nitropropene (**13**)[19] gave essentially a quantitative yield of the dibenzyloxyamine **14**[12] which as its hydrochloride salt **15** was hydrogenolyzed[20] to yield crystalline (±)-α-methyldopamine hydrochloride [(±)-**2**·HCl].[14]

The model recovery experiment was carried out by adding a small amount of (±)-**2**·HCl to control urine (pH adjusted to 2) obtained from a volunteer. Diazomethane methylation of a methanol extract of the lyophylized urine provided, after work-up, a base fraction which by glpc analysis gave a peak identical in retention time with synthetic (±)-**11**. Furthermore, chemical ionization mass spectrometry (CIMS) of this base fraction contained an ion at *m/e* 196 corresponding to the protonated molecular ion of **11**. After treatment with the PFBPI reagent, the glpc tracing showed two equally intense peaks corresponding to the diastereomeric amides **8a** and **8b** as obtained from direct derivatization of racemic **11**.

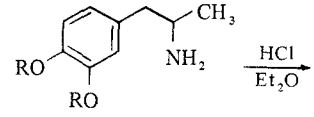
Having shown that both the methylation-isolation and

‡ Commercially available from Aldrich Chemical Co., Milwaukee, Wis.

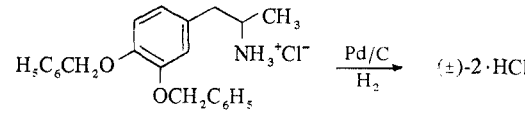


9, R = CH_3
12, R = $CH_2C_6H_5$

10, R = CH_3
13, R = $CH_2C_6H_5$

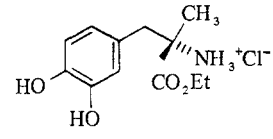


(±)-**11**, R = CH_3
(±)-**14**, R = $CH_2C_6H_5$



15

the detection-identification of each enantiomorph of **11** are possible, urine from a patient on α-methyldopa (**1**) medication was examined in a similar fashion. During the period in which urine was being collected, the patient (TLS) was taking orally α-methyldopa (**1**) and intravenously methyldopate HCl [ethyl (*S*)-(−)-3-(3,4-dihydroxyphenyl)-2-methylalaninate hydrochloride, **16**].



(*S*)-(−)-**16**

The same lyophylization, methylation, and extraction procedures were followed as were used with the control urine. Glpc analysis of the base fraction showed the desired peak for methylated α-methyldopamine although the tracing itself was quite different from that observed with the control urine. Low-resolution CIMS of the crude base fraction gave an ion at *m/e* 196 which on high resolution gave the molecular formula of $C_{11}H_{18}NO_2$ corresponding to compound **11** + H. Derivatization of this base fraction with PFBPI gave a glpc tracing in which the retention time of the major peak exactly corresponded to that observed for **8a**, the (*S*:*S*)-amide, obtained from authentic (*S*)-(+)-amine and PFBPI. Since the region of the tracing where the (*R*:*S*)-amide **8b** would be expected appeared to consist of more than one substance, the parent base was collected off the glpc and analyzed after PFBPI derivatization. Glpc analysis of this sample gave only the peaks corresponding to amides **8a** and **8b** with relative areas of 11:1. Exact mass determination of the *m/e* 486 peak observed in the CIMS of this amide reaction mixture gave a mass corresponding to the molecular formula $C_{23}H_{23}F_5N_2O_4$ (amides **8a** and **8b**).

From these data the absolute configuration of the majority of the α-methyldopamine obtained metabolically from α-methyldopa is unambiguously established as *S*-(+). The origin of the small amount of the (*R*)-(−)-amine **7b** remains unknown. Possible sources include partial racemization of **2a** during work-up,[12] the presence of some (*R*)-amino acid or (*R*)-amino ester in the drugs taken by TLS coupled with decarboxylation to **7b**, or incomplete stereospecificity in the decarboxylation of **1**. It should also be pointed out that the 1-(3,4-dimethoxyphenyl)-2-aminopropanes **7a** and **7b** isolated and identified in these experiments could also arise from diazomethane methylation of the 1-

(3-methoxy-4-hydroxyphenyl)-2-aminopropanes **17a** and **17b** since O-methylation is a known metabolic reaction in this series.[1f] These problems are currently under investigation.

Experimental Section

Melting points were determined in a Thomas-Hoover Uni-Melt stirring oil capillary tube melting point apparatus and are uncorrected. All boiling points are also uncorrected. The infrared spectrum was measured with a Perkin-Elmer Model 337 infrared spectrophotometer. Proton magnetic resonance spectra were determined at 60 MHz with a Varian Model A-60A pmr spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to either a TMS or a sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) internal standard. In the presentation of the pmr spectra, the following notations are used: s = singlet, d = doublet, m = multiplet, and comp m = complex multiplet. The mass spectra were obtained with an Associated Electronic Incorporated Model MS 902 double focus mass spectrometer equipped with a direct inlet system and modified to do chemical ionization mass spectra.§ In all cases isobutane was used as the reactant gas. Lyophylizations were performed in a Virtis Freeze Dry Unit. Gas-liquid partition chromatography was performed on a Varian Aerograph Model 2100-00 life sciences gas chromatograph. A U-shaped 6 ft × 0.25 in. × 2 mm i.d. Pyrex column packed with 3% SE-30 on acid-washed, DMCS-treated Chromosorb W was used under two sets of conditions: (a) column temperature 150°, chart speed 6.4 mm/min, attenuation 8×10^{-11}; and (b) column temperature 190°, chart speed 2.5 mm/min, attenuation 1×10^{-11} or 8×10^{-12}.

Sample collection was performed using 3% SE-30 on Chromosorb W in a 5.5 ft × 2 mm i.d. Pyrex column equipped with a 10:1 effluent splitter at 125°. The refractive index was determined on a Model 1300 Spencer refractometer. The microanalysis was performed by the Microanalytical Laboratory, University of California, Berkeley, Calif.

Diazomethane. The diazomethane used in esterifications and O-methylations was prepared by the base decompositon of Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide).[21] When large amounts of diazomethane were required, it was prepared in individual 3-g batches.

(±)-1-(3,4-Dibenzyloxyphenyl)-2-aminopropane (14). Into a 1000 ml, three-neck flask equipped with a magnetic stirrer, large and small pressure equalizing dropping funnels, and N_2 inlet and outlet tubes were placed freshly dried and distilled THF (300 ml) and $LiAlH_4$ (11.45 g, 302 mmol). The system was maintained at 3°, purged with liquid N_2–dried N_2, and kept under a positive pressure of N_2 at all times. The AlH_3[16] (9.06 g, 302 mmol) was generated by the very slow addition of 100% H_2SO_4 (14.8 g, 151 mmol) to the $LiAlH_4$–THF suspension. Following an additional 30 min, a solution of 1-(3,4-dibenzyloxyphenyl)-2-nitropropene[19] (**13**, 25.65 g, 68.5 mmol) in 400 ml of dry THF was added over a 2-hr period. The resulting mixture was allowed to warm to room temperature and stirred for an additional 10 hr. The work-up procedure[22] involved the slow addition of 11.5 ml of distilled H_2O followed by 11.5 ml of 15% NaOH and 34.5 ml of distilled H_2O. To complete salt formation 22.0 ml of 15% NaOH was added. The mixture was stirred for 1 hr and the salts were filtered off. The filter cake was leached with Et_2O (200 ml) for 30 min and filtered. The ethereal solution was combined with the original THF filtrate and the resulting solution dried over K_2CO_3. Removal of the drying agent, followed by concentration *in vacuo*, gave 23.8 g (68 mmol, 100%) of 1-(3,4-dibenzyloxyphenyl)-2-aminopropane (**14**) as a very low melting crystalline solid. Pmr analysis indicated the crude product to be essentially pure. An analytical sample was obtained by two molecular distillations [155° (0.005 mm) and 180° (0.05 mm)] which yielded a clear colorless oil that crystallized on standing: mp 33.5–35.5° (lit.[12] mp ~35°, isoamyl ether); ir $\nu_{max}^{CHCl_3}$ 3360 cm^{-1}(NH); pmr ($CDCl_3$) δ 1.01 (d, J = 6 Hz, CH_3), 1.22 (bd s, NH_2), 1.95–3.44 (ABC pattern,[23] $ArCH_2CH$), 5.09 (s, OCH_2Ar), 5.11 (s, OCH_2Ar), and 6.55–7.60 (comp m, 13 H, arom H). *Anal.* ($C_{23}H_{25}NO_2$) C, H, N. The HCl salt of **14** was obtained by passing HCl through a solution of the free base **14** (4.53 g, 13.04 mmol) in 100 ml of dry Et_2O. After cooling to 0° the Et_2O was decanted away leaving a white solid which was crystallized twice from EtOH–Et_2O to give 4.17 g (83.3%) of pure **(±)-1-(3,4-dibenzyloxyphenyl)-2-aminopropane hydrochloride (15),** mp 133–134° (lit.[12] mp 135–136.5°, CH_3CN).

(±)-α-Methyldopamine Hydrochloride ((±)-2·HCl). The above hydrochloride **15** (2.00 g, 5.22 mmol) was hydrogenolyzed at atmospheric pressure and room temperature over 10% Pd/C (601 mg) in 60 ml of dry MeOH. After 35 min, 283 ml of H_2 uptake was observed (theoretical, 234 ml). The catalyst was filtered and the clear, colorless solution concentrated to dryness *in vacuo* to give a foamy solid material, 1.15 g, which was recrystallized from EtOH–Et_2O to yield slightly colored crystals (651 mg) melting at 192–194° (lit.[14] mp 194°, EtOH–Et_2O). A second crop of crystals (184 mg) melted at 188–193°: combined yield 835 mg (78.7%); pmr (D_2O with 1% DSS) δ 1.37 (d, J = 6 Hz, CH_3), 2.75–3.09 (comp m, $ArCH_2$), 3.09–3.88 (comp m, CHN), and 6.69–7.12 (comp m, 3 H, arom H).

(±)-1-(3,4-Dimethoxyphenyl)-2-aminopropane (11). Following the procedure given for the preparation of **14**, 1-(3,4-dimethoxyphenyl)-2-nitropropene[15b,17] (**10**, 10.0 g, 44.8 mmol) was reduced with AlH_3 (5.38 g, 179 mmol) in THF. Pmr analysis of the crude light brown oil (8.6 g, 98%) indicated the presence of only the desired product. Short-path distillation gave 7.50 g (84.6%) of a colorless oil: bp 70–72° (0.01 mm) [lit.[15b,#] bp 95–97° (0.05 mm)]; $n^{25}D$ 1.5321; pmr ($CDCl_3$) δ 1.11 (d, J = 6.5 Hz, CH_3), 1.46 (s, NH_2) 2.22–3.42 (comp m, $ArCH_2CH$), 3.86 (s, OCH_3), 3.89 (s, OCH_3), and 6.63–6.95 (m, 3 H, arom H); glpc retention time (conditions a) 3.3 min.

Detection and Identification of (±)-1-(3,4-Dimethoxyphenyl)-2-aminopropane (11) in Control Human Urine to Which Has Been Added (±)-α-Methyldopamine Hydrochloride [(±)-2·HCl]. The pH of 340 ml of control human urine to which had been added (±)-α-methyldopamine HCl [(±)-**2**·HCl, 410.6 mg] was adjusted to 2 with *p*-TsOH (3.48 g). The residue obtained after lyophylization was leached with 250 ml of dry MeOH for 3 hr at room temperature and the resulting extract cooled to 3° and filtered through Celite. An excess of diazomethane (*ca.* 9 g) in Et_2O was slowly added with stirring to the cooled filtrate. After stirring at room temperature overnight, the reaction mixture was concentrated to dryness *in vacuo* to yield 13.77 g of a dark brown semisolid. The residue in 250 ml of 3 *N* HCl was extracted (5 × 100 ml) with $CHCl_3$ and the combined $CHCl_3$ extracts were backwashed with 100 ml of 3 *N* HCl. The combined aqueous layers at 3° were made to pH 12 with 3 *N* NaOH and the resulting solution was extracted with (6 × 100 ml) $CHCl_3$. The combined $CHCl_3$ extracts were washed with (3 × 200 ml) H_2O, filtered, and dried over K_2CO_3. Concentration *in vacuo* of the resulting solution gave 90 mg of a brown solid residue which upon glpc analysis (conditions a) showed three major peaks with retention times 3.3, 4.0, and 5.1 min and at least eight minor peaks. The 3.3-min peak corresponded to the retention times of authentic (±)-1-(3,-4-dimethoxyphenyl)-2-aminopropane (**11**). CIMS at 30° of this crude residue gave peaks with *m/e* 196 (M + 1), 179 (M + 1 − NH_3), and 163.4 (the metastable ion for 196 → 179).

Detection and Identification of 1-(3,4-Dimethoxyphenyl)-2-aminopropane in the Urine of a Patient Receiving (*S*)-(−)-α-Methyldopa. Urine (870 ml) obtained from a severely hypertensive patient receiving daily (*S*)-(−)-α-methyldopa (**1**, 1.5 g) orally and (*S*)-(−)-α-methyldopa ethyl ester (**16**, 2.0 g) intravenously was immediately acidified to pH 1.8 with concentrated HCl. Following exactly the procedure used in the above-described control study yielded 210 mg of crude, brown-colored, oily residue. The glpc tracing (conditions a) was very different from that obtained from the control and consisted of one very large peak with a retention time of 12.0 min, three intermediate sized peaks with retention times of 3.3, 9.1, and 16.5 min, and at least 11 minor peaks. Peak-enhancement experiments indicated the 3.3-min peak corresponded to the desired aminopropane **11**. The CIMS at room temperature of this crude residue exhibited peaks at *m/e* 196 (M + 1), 179 (M + 1 − NH_3), and 163.4 (the metastable ion for 196 → 179). Mass determination on the *m/e* 196 peak gave an exact mass of 196.1334 (calcd for $C_{11}H_{18}O_2N$, 196.1337). Synthetic 3,4-dimethoxyphenylacetone (exact mass, 195.1021) was used as the internal standard. Only the $C_{11}H_{18}O_2N$ peak was observed at *m/e* 196.

The material corresponding to the 3.3-min peak was collected on glpc using 10:1 effluent stream splitter. The material crystallized in the 3-mm collection tube on standing.

Diastereomeric Amide Formation and Detection Using (*S*)-(−)-*N*-Pentafluorobenzoylprolyl-1-imidazolide (6). General Procedures. Following the procedure of Matin, *et al.*,[11] small samples (0.05 mmol) of amine hydrochloride or hydrosulfate were placed in a 2-g opti-clear vial along with 1 *N* NaOH (1 ml) and C_6H_6 (2 ml). The mixture was shaken vigorously and the C_6H_6 layer pipetted into

§ Details of the instrumental modifications will be published elsewhere by W. A. Garland, R. J. Weinkam, and W. F. Trager.

#Reference 15a gives bp 166–168° (20 mm) and ref 14 gives bp 124–130° (1.5 mm).

another vial containing a small amount of anhydrous Na_2SO_4. After shaking, this mixture was filtered into a third vial containing (*S*)-(−)-*N*-pentafluorobenzoylprolyl-1-imidazolide (**6**, 0.009 mmol). The vial was tightly stoppered and shaken with very gentle warming until the solid imidazolide dissolved. Glpc (conditions b) samples were taken directly from this vial. When the basic components of diazomethane treated lyophylizates were employed, the above procedure was followed except that the ratio of base to imidazolide was *ca.* 1:1. In the cases where the free amine was directly available, *ca.* 0.05 mmol was taken up in 2 ml of dry C_6H_6 and added directly to the imidazolide vial. The results of these analyses are discusssed in the text.

Mass determination on the *m/e* 486 peak of the amide reaction mixture from the collected metabolites gave an exact mass of 486.1583 (calcd for $C_{23}H_{23}F_5N_2O_4$, 486.1578).

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Ring D Bridged Steroid Analogs. 11.[1] The High Clauberg Activity of 19-Nor-14α,17α-ethano-4-pregnene-3,20-dione†

A. J. Solo* and J. N. Kapoor

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14214.
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3β-Acetoxy-14α,17α-etheno-5-pregnen-20-one (**1**) was converted to 14α,17α-ethano-4-pregnene-3,20-dione (**9**) and to 19-nor-14α,17α-ethano-4-pregnene-3,20-dione (**8**). On subcutaneous administration **9** was found to have 2.2 times the Clauberg activity of its 15′-dehydro analog **10**. **8**, which lacks the 10β-methyl of **9**, showed a further 13.6-fold increase in Clauberg activity and is 17.6 times as active as progesterone. The possible significance of these results is discussed.

Recently, in order to account for a number of seemingly anomolous structure-activity effects, and especially to explain the surprisingly low Clauberg activity of 14α,17α-etheno-4-pregnene-3,20-dione (**10**), we proposed[2] an extension of Ringold's hypothesis[3] on the nature of binding of progesterone to its receptor. Essentially, our argument is that a two-atom bridge between the 14α and 17α positions of progesterone causes the 17-substituent (OH or acetyl side chain) to be deflected toward the α side of the molecule. Because the 17-substituent appears to interact strongly with the Clauberg receptor, the effect of this deformation is to increase the apparent bulk of all β substituents. However, the 10β-methyl group seems to be too large for optimum fit to the receptor, even in the case of progesterone. The result of any such deformation is therefore to decrease the binding affinity between the hormone analog and the Clauberg receptor. If our argument is correct, removal of the 10β-methyl group from a 14α,17α-etheno- (or ethano-) bridged progesterone analog should result in a larger than normal increase in Clauberg activity. We have already demonstrated this effect for 14α,17α-ethano-4-

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