Investigation of Some Methylated Products Obtained by Reaction of β-3,4-Dihydroxyphenylalanine (Dopa) with Diazomethane in Methanol–Ether

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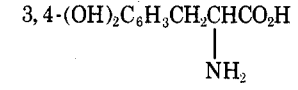
Received April 16, 1975

β-(3,4-Dihydroxyphenyl)alanine (1), usually referred to as Dopa, is a primary precursor in both catecholamine[1] and melanin biosynthesis.[2] The levo isomer of 1 is also current-

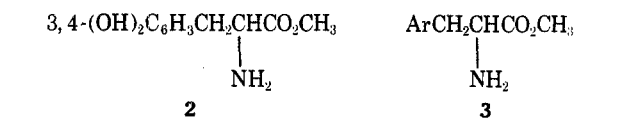


1

ly therapeutically useful in treating Parkinson's disease.[3] As a result of other studies in our laboratory, and because of the potential physiological significance of methylated analogs of 1, a study of the alkylation of Dopa with diazomethane was undertaken. Previous studies of the reactions of some amino acids with diazomethane were conducted in moist ether, absolute ethanol, or ethanol–water.[4] In the present study, reactions were conducted in methanol–ether (1:1). Such a common solvent mixture slightly enhances solubility of the amino acid (relative to ether alone) and enables a study of the reactions of three functional groups in a nonaqueous medium.

Reactions were performed by suspending 0.05 mmol (10 mg) of 1 in 5 ml of methanol and adding 5 ml of a 0.2 *M* solution of diazomethane (20 molar excess) in ether. Dopa was initially relatively insoluble in the solvent system, but solution was effected between 4 and 8 hr. In order to maintain a constant temperature, to minimize loss of reagent and solvent, and to avoid artifacts due to light, the reactions were carried out at 4°C, in a glass stoppered flask, in the dark. The reaction times varied from 5 min to 24 hr and the reaction products were studied by comparison of both TLC and VPC with known synthetic products. In several reactions, analyses of products were confirmed by ir spectra and GC–MS data.

The treatment of Dopa with diazomethane led to eventual alkylation of each functional group present, i.e., carboxyl, phenolic, and amino. With respect to time, the appearance of each compound was in the order of increased methylation, i.e., 2 was detected before 3, 3 before 4, and 4 prior to 5. Interestingly, traces of the trans cinnamate methyl ester (6) appeared at 24 hr. At the end of 24 hr, the reaction solution was clear and almost colorless, and at times longer than 24 hr, no further change was observed in the reaction products, an indication that all of the diazomethane reacted and/or evaporated. Essentially all of the reacted Dopa could be accounted for by compounds 3–6 at the 24-hr time period.



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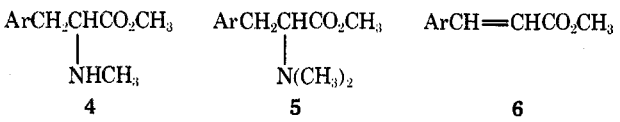


4 5 6

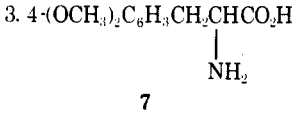
Ar = 3,4-dimethoxyphenyl

Although Dopa is quite insoluble in methanol–ether, esterification commenced instantaneously as evidenced by the formation of 2 within 5 min. After 8 hr, essentially all of 1 had reacted. The phenolic groups were rapidly methylated and small amounts of 3 were detected at 5 min. At the end of 16 hr, all traces of catechol (2) had disappeared. The insolubility of 1 is apparently the prime cause for the slow rate of esterification and methyl ether formation, since both carboxyl and phenolic methylation of solubilized substrates are usually quite rapid.

In the TLC observations, at least three additional spots appeared at R_f values between that of 2 and 3. These spots were observed as early at 5–10 min, reached maximum intensity at 30–60 min, noticeably decreased after 2 hr, and completely disappeared at the end of 16 hr. Attempts to isolate the compounds causing these spots after a large-scale reaction were fruitless. These substances could be unstable intermediates or other products which may have also interfered with VPC analysis. Attempts to determine which of the phenolic groups was first methylated were also precluded by the fact that the methyl esters of 3-*O*-methyl-Dopa and 4-*O*-methyl-Dopa appeared at R_f values very close to that of the unidentified spots mentioned above.[5]

The amino group was the slowest to react and N-methylation occurred only after the carboxyl and phenolic functions had begun to react. However, N-alkylation was observed before complete esterification was achieved. The monomethyl analog 4 was detected within 1 hr and traces of the *N,N*-dimethyl derivative 5 were observed at the 2-hr time period. That the reaction pathway does not involve a continuous direct methylation of the amino esters was demonstrated by the fact that no reaction occurred at the end of 24 hr when methanolic solutions of 3 or 4 were treated with ethereal diazomethane. These results could be explained by assuming small amounts of the dipolar ions continuously generated from Dopa and which can be slowly alkylated to the N-methylated zwitterions. In the present study, the intermediate dipolar N-methylated amino acids were not detected by TLC methods when the reaction was conducted in the absence of water. However, when the reaction was conducted in moist ether, the amino acids of 3, 4, and 5 were readily detectable. These observations are also consistent with that of Kuhn and co-workers, who demonstrated that when water was present in ether solutions of diazomethane, N-methylated dipolar ions were formed.[4] In absolute ethanol using dry diazomethane, the esters were not converted into dipolar ions.[4b] With increasing amounts of water, greater quantities of the dipolar ions were formed, partly owing to the hydrolysis of the amino methyl esters.

The cinnamate ester (6), however, was not obtained from the uncharged amino methyl esters. To ensure that loss of ammonia from 3 or dimethylamine from 5 to give 6 was not base catalyzed, a mixture of 3 and 5 in MeOH–Et_2O for 24 hr also gave no reaction. When the free acid form, 7, was



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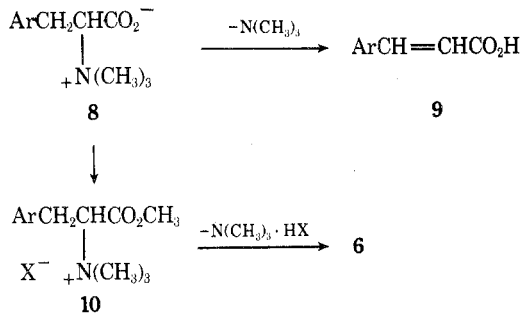
treated with diazomethane, the formation of 3, 4, 5, and 6 was observed.

Two plausible pathways leading to the formation of the cinnamate ester (6) may be envisaged. Elimination of trimethylamine from 8 gives the unsaturated acid 9, which upon additional methylation may produce 6. Similarly, Hofmann elimination of the permethylated analogue 10 would furnish 6 directly.[6]

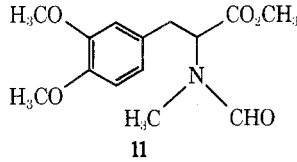
In no case could the trimethyl betaine (8) be isolated. Even when water was used in place of methanol, the betaine could not be obtained. Several different methods of



Ar = 3,4-dimethoxyphenyl

alkylation, including the use of dimethyl sulfate and methyl iodide, failed to produce the betaine from **1**. Kuhn had isolated the trimethyl betaine of phenylalanine in 39% yield (plus a 50% yield of amino ester)[4a] but no mention of a betaine was made when tyrosine was made to react with diazomethane.[7] The presence of alkoxy groups on the aromatic nucleus may enhance the formation of the conjugated cinnamyl system.

The syntheses of compounds **2–6** were achieved without much difficulty, but provided some interesting observations. The formamide **11** was utilized in the preparation of



11

4. In the NMR of **11**, the methyl protons of the carbomethoxy group and the proton of the formyl group each appeared as two peaks of approximately equal height, with the former at δ 3.73 and 3.75 and the latter at δ 7.84 and 8.00, respectively. These results may be explained by assuming two different configurations, i.e., the cis and trans rotamers of **11**, which are quite common in N-substituted formamides.[8] Similar observations in the NMR have been previously reported for esters of substituted formamides.[9] Thus, the protons giving rise to two peaks exist in different chemical environments having a population of approximately 50% each. One might also expect to observe two peaks for the *N*-methyl protons as well. Although two distinct peaks were not observed, the NCH_3 peak at δ 2.86 had a break in it and was not as sharp as the NCH_3 peaks of **4** and **5**. Addition of deuteriobenzene clearly separated the *N*-methyl peaks, resulting in a significant upfield shift of the methyl trans to the amide carbonyl.[8b]

The *N,N*-dimethyl analogue **5** could be prepared in quantitative yield by catalytic reductive alkylation of **3**.[10] It was essential that the free base was used, since the hydrochloride of **3** apparently gave the isoquinoline derivative, the NMR of which was similar to that of analogous compounds previously reported.[11]

Finally, the hydrochlorides of **3, 4,** and **5** were converted to analytical samples of the free amines by passing methanolic solutions of the hydrochlorides through an anion (OH^-) exchange column and elution with methanol. Very little ester hydrolysis was observed in this very useful but apparently seldom used procedure[12] for conversion of the amine salt to the free amino ester.

Experimental Section

Ir spectra were obtained with $CHCl_3$ solutions recorded on a Perkin-Elmer Infracord Model 137B. NMR spectra were obtained from a Varian 60-MHz instrument with $CDCl_3$ solutions (except where noted otherwise) using Me_4Si as an internal standard. Reported NMR data are expressed in δ units. Mass spectra were obtained using a LKB Model 9000 gas chromatograph–mass spectrometer. VPC was performed employing a Varian Model 1860 unit in the fid mode; column conditions 6 ft × 0.125 in. stainless steel 3% OV-1 on Supelcoport, 160°, 30 ml/min He flow. Recorded R_f values are the averages of at least five runs on silica gel GF plates (250 μm thick, Analtech) with C_6H_6–CH_3OH (6:1) as a solvent. Prior to use, the plates were dried at 120°C for 2 hr, then transferred immediately to a desiccator containing Drierite. For comparison on TLC the amino acids of **3, 4,** and **5** were obtained by hydrolysis of the related esters. Melting points were observed in capillaries in a Thomas-Hoover apparatus and are uncorrected. Purified compounds gave one spot on TLC, and one peak on VPC. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Dopa was purchased from Nutritional Biochemical Corp. and was found to be chromatographically (TLC) pure.

General Procedure for the Reactions of Dopa with Diazomethane. An ethereal solution of diazomethane was prepared from *N,N'*-dimethyl-*N,N'*-dinitrosoterephthalamide.[13] Titration with benzoic acid[14] indicated the diazomethane solution to be 0.2 *M*. To a 50-ml round-bottom flask containing a Teflon-coated magnetic stirring bar and a suspension of finely powdered **1** (10 mg, 5.1×10^{-2} mmol) in absolute methanol (5.0 ml) was added 0.2 *M* diazomethane–ether (5.0 ml, 20-fold excess). The reaction flask was stoppered lightly and placed in the cold room at 4° in the dark. Single reaction runs were made at 5, 10, 15, 20, 30, and 45 min. Duplicate studies were made at 1, 2, 4, 8, 16, and 24 hr. The solvent was removed at the rotary evaporator at 25° or less and the reaction residue was treated with 1.0 ml of absolute methanol. Both TLC and VPC results were compared with standard solutions prepared from the synthetic compounds. Identification of the amino acid methyl esters was also confirmed in a few reactions by gas chromatography–mass spectrometry, and by observing the decrease of ir absorbance of the N–H bond (ca. 3300–3400 cm^{-1}) as time progressed.

***dl*-β-(3,4-Dihydroxyphenyl)alanine Methyl Ester (2).** The methyl ester hydrochloride of **1** was prepared in greater than 95% yield by either of two methods: (a) refluxing in CH_3OH saturated with HCl gas for several hours or (b) gentle refluxing for 1–2 hr in $SOCl_2$–CH_3OH. The free amine was liberated from the hydrochloride using the method of O'Neill et al.[15] Recrystallization from $CHCl_3$–petroleum ether (bp 30–60°) gave white crystals, mp 124–126° (lit.[15] 126°), R_f 0.32.

***dl*-β-(3,4-Dimethoxyphenyl)alanine Methyl Ester (3).** Using the procedure of Schrecker and Hartwell,[16] *N*-formyl-β-(3,4-dimethoxyphenyl)alanine was refluxed in CH_3OH saturated with gaseous HCl to give **3** HCl in 97% yield. Recrystallization from CH_3OH–Et_2O gave white crystals, mp 183.5–184° dec (lit.[16] 185–186° dec). A solution of the hydrochloride (551 mg, 2.0 mmol) in a minimum amount of methanol was placed on a chromatography column (1 × 30 cm) containing Bio-Rad Dowex Ag-1X-8 (200–400 mesh, OH^- form) and eluted with CH_3OH.[12] After 1 ml of forerun, 2-ml fractions were collected and monitored by TLC. Fractions 5–13 were combined to yield 393 mg (82%) of the free amine **4** as a straw-colored oil: R_f 0.47; ir 3400, 3340 (NH_2), 1735 cm^{-1} (C=O); NMR δ 1.50 (broad, NH_2), 2.98 (d, 2, CH_2), 3.2–2.4 (m, 1, CH), 3.71 (s, 3, CO_2CH_3), 3.86 (s, 6, $ArOCH_3$), 7.73–7.80 (m, 3, ArH); *m/e* 239 (M^+), 180 ($M^+ - CO_2CH_3$), 151 ($M^+ - H_2NCHCO_2CH_3$). Anal. Calcd for $C_{12}H_{17}NO_4$: C, 60.24; H, 7.16; N, 5.85. Found: C, 59.78; H, 7.03; N, 5.60.

***dl*-*N*-Formyl-*N*-methyl-β-(3,4-dimethoxyphenyl)alanine Methyl Ester (11).** The preparation of **11** was conducted similar to a procedure described by Olsen.[17] To a solution of *N*-formyl-β-(3,4-dimethoxyphenyl)alanine[16] (1.012 g, 4.0 mmol) in 20 ml of freshly distilled anhydrous DMF was added 1.2 ml (19.6 mmol) of CH_3I, followed by freshly prepared Ag_2O (2.784 g, 12.0 mmol). The reaction mixture was stirred for 12 hr at room temperature and filtered, and the solid was washed twice with 1–2 ml of DMF. $CHCl_3$ (100 ml) was added to the filtrate and a slight precipitate formed. The filtrate was transferred to a separatory funnel and washed with 5% aqueous KCN (2 × 20 ml) and distilled H_2O (4 × 20 ml or until neutral to pH paper), then dried ($MgSO_4$) overnight. The solution was decanted and evaporated (under 40°) to an oil. Last traces of solvent were removed at 35° (0.5 mm), giving 760 mg of yellow-brown oil. White crystals could be obtained from the oil in either of two ways: (a) addition of EtOAc to about twice the volume of oil and storage of the solution in the refrigerator for several days, or (b) chromatography on silicic acid, eluting with CH_2Cl_2 and then CH_2Cl_2–CH_3OH (9:1). Recrystallization from EtOAc gave mp 92.5–105°.[18] Attempts to recrystallize from at least 13 different solvent systems either failed to give crystals or did not fur-

ther affect the melting point range: ir 1735 (ester C=O), 1665 cm^{-1} (amide C=O); NMR δ 2.86 (s, 3, NCH_3), 3.20 (d, 2, $ArCH_2$), 3.4–3.6 (m, 1, CH), 3.73 and 3.75 (s, s, 3, CO_2CH_3), 3.84 (s, 6, $ArOCH_3$), 6.67–6.83 (m, 3, ArH), 7.84 and 8.00 (s, s, 1, CHO); upon addition of C_6D_6 all peaks shifted upfield with separation of the NCH_3 and $ArOCH_3$ signals into each of two distinct peaks; *m/e* 281 (M^+), 222 ($M^+ - CO_2CH_3$), 151 [$M^+ - CH_3(CHO)NCHCO_2CH_3$, base]. Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98. Found: C, 59.54; H, 6.61; N, 4.87.

***dl*-*N*-Methyl-β-(3,4-dimethoxyphenyl)alanine Methyl Ester (4).** Using the procedure above for the preparation of **3**, 155 mg (0.05 mmol) of **11** was converted to the hydrochloride of **4**. Recrystallization from C_6H_6–CH_3OH gave 92 mg (58%) of **4** HCl as a white solid (one spot on TLC). Utilizing the ion-exchange procedure described above, 66 mg of free amine was obtained as a clear oil: R_f 0.50; ir 3300 (NH), 1725 cm^{-1} (C=O); NMR δ 1.60 (broad, NH), 2.35 (s, 3, NCH_3), 2.90 (d, 2, CH_2), 3.28–3.50 (m, 1, CH), 3.67 (s, 3, CO_2CH_3), 3.87 (s, 6, $ArOCH_3$), 6.70 (m, 3, ArH); *m/e* 253 (M^+), 194 ($M^+ - CO_2CH_3$), 151 ($M^+ - H_3CNHCHCO_2CH_3$), 102 ($H_3C^+NH{=}CHCO_2CH_3$, base). Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.67; H, 6.99; N, 5.45.

***dl*-*N*,*N*-Dimethyl-β-(3,4-dimethoxyphenyl)alanine Methyl Ester (5).** To a solution of **3** (95.6 mg, 0.4 mmol) in 2 ml of CH_3OH were added paraformaldehyde (85 mg), 10% Pd/C (50 mg), and anhydrous $MgSO_4$ (50 mg). The mixture was subjected to a reductive alkylation at 45° similar to the procedure of Bowman and Stroud[10] to give **5** (107 mg, 100%) as a colorless, clear oil. An analytical sample of the free amine was prepared by making the solid hydrochloride, and recrystallization, followed by the ion-exchange procedure described above: R_f 0.60; ir 1725 cm^{-1} (C=O); NMR δ 2.38 (s, 6, NCH_3), 2.94 (dd, 2, CH_2), 3.28–3.47 (m, 1, CH), 3.59 (s, 3, CO_2CH_3), 3.84 (s, 6, $ArOCH_3$), 6.75 (s, 3, ArH); *m/e* 267 (M^+), 151 [$M^+ - (H_3C)_2N{=}CHCO_2CH_3$], 116 [$(H_3C)_2{}^+N{=}CHCO_2CH_3$, base]. Anal. Calcd for $C_{14}H_{21}NO_4$: C, 62.90; H, 7.92; N, 5.24. Found: C, 62.81; H, 7.86; N, 5.15.

When the hydrochloride of **3** was treated under identical conditions stated above, the major product appeared to be the isoquinoline analogue. Ion exchange produced an oil: NMR δ 2.53 (s, 3, NCH_3), 3.04 (d, J = 6 Hz, 2, $ArCH_2CHCO_2$–), 3.54 (t, J = 6 Hz, 1, CH), 3.72 (s, 3, CO_2CH_3), 3.83 (s, 8, two $ArOCH_3$ + $ArCH_2N$), 6.54 (s, 1, ArH), 6.60 (s, 1, ArH).

3,4-Dimethoxycinnamic Acid Methyl Ester (6). The cinnamate ester was prepared from the free acid by gentle refluxing in $SOCl_2$–CH_3OH, mp 60–62° (lit.[19] 68–69°), R_f 0.88.

Acknowledgments. This investigation was supported in part by NIH Grant CA-11959. Mass spectra were obtained by Mr. John Naworal on an LKB-9000 supported by NIH RR-00273. Appreciation is also extended to James Ramsey (supported by ACS Project Seed) and Donald Evans for some technical assistance. 3-*O*-Methyl-Dopa and 4-*O*-methyl-Dopa were kindly provided by Dr. Sidney Teitel of Hoffmann-La Roche, Inc.

Registry No.—**1**, 63-84-3; **3**, 56771-16-5; **3** HCl, 56771-17-6; **4**, 56771-18-7; **5**, 56771-19-8; **5** HCl, 56771-20-1; **11**, 56771-21-2; *N*-formyl-β-(3,4-dimethoxyphenyl)alanine, 53053-93-3; 2-methyl-3-carbomethoxy-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, 56771-22-3; diazomethane, 334-88-3.

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(5) With the aid of three different spray reagents, the unidentified substances appearing at R_f values between that of **2** and **3** afforded different colors than that observed by spraying samples of 3-*O*-methyl-Dopa and 4-*O*-methyl-Dopa. Based on the overall observations, it appears that very small amounts, if any, of the monomethoxy methyl esters are present during the reaction.
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