Peyote and Related Alkaloids XVI: Synthesis of 3,4,5-Trimethoxyphenylalanine, an Amino Acid Analog of Mescaline

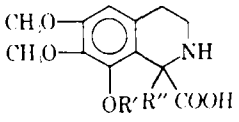
MANOHAR L. SETHI, G. SUBBA RAO*, and GOVIND J. KAPADIA▲

Abstract □ Contrary to an earlier literature report, acid hydrolysis of diethyl α-acetamido-α-3,4,5-trimethoxybenzylmalonate during the synthesis of 3,4,5-trimethoxyphenylalanine has been shown to lead to a mixture of products. The major hydrolysis products were isolated and identified by NMR and mass spectrometry as 3,4,5-trimethoxyphenylalanine (34%), 4-hydroxy-3,5-dimethoxyphenylalanine (13%), 3-hydroxy-4,5-dimethoxyphenylalanine (8%), and 3,4-dihydroxy-5-methoxyphenylalanine (5%). Under basic conditions, the acetamidomalonate ester as well as its formamido analog, obtained by the condensation of trimethoxybenzyl chloride and diethyl formamidomalonate, gave low yields (5–7%) of the required trimethoxyphenylalanine. However, no phenolic side-products were formed under basic conditions. An efficient alternative synthesis of 3,4,5-trimethoxyphenylalanine has been accomplished; it involves the condensation of hydantoin with trimethoxybenzaldehyde followed by hydrogenation and basic hydrolysis of the resulting 5-(3,4,5-trimethoxybenzylidine)hydantoin. GC–mass spectrometric examination of the peyote amino acid fraction gave no evidence for the presence of trimethoxyphenylalanine and other trioxygenated phenylalanine analogs obtained during these synthetic studies.

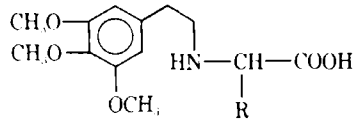
Keyphrases □ 3,4,5-Trimethoxyphenylalanine (mescaline analog)—synthesis □ Peyote and related alkaloids—synthesis of 3,4,5-trimethoxyphenylalanine (amino acid analog of mescaline) □ Mescaline derivatives—synthesis of 3,4,5-trimethoxyphenylalanine

In recent studies on peyote [*Lophophora williamsii* (Lem.) Coult.] constituents, the authors examined the amino acid fraction of the cactus and reported the occurrence of several novel nonproteinic amino acids (1–5). These include four C_1-carboxytetrahydroisoquinolines (I–IV), two *N*-alkyl-α-amino acids (V and VI), and a pyrrole-2-carboxylic acid (VII).

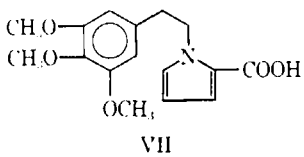
To identify additional unknown constituents present in the peyote amino acid fraction (3), the possible occurrence of trioxygenated analogs of phenylalanine was considered. Since mescaline (3,4,5-trimethoxyphenethylamine) is the major alkaloid present in the peyote cactus, synthesis of its amino acid analog, 3,4,5-trimethoxyphenylalanine (VIII), was undertaken to check for its natural occurrence. This amino acid analog of mescaline was also needed to evaluate its hallucinogenic and other biological activities.



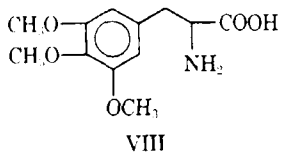
I: R′ = R″ = H
II: R′ = H, R″ = CH$_3$
III: R′ = CH$_3$, R″ = H
IV: R′ = R″ = CH$_3$



V: R = H
VI: R = CH$_3$



VII



VIII

RESULTS AND DISCUSSION

Synthesis by Literature Method—Synthesis of 3,4,5-trimethoxyphenylalanine (VIII) was reported previously by Acheson *et al.* (6). The procedure consists of preparation of the key intermediate, diethyl α-acetamido-α-3,4,5-trimethoxybenzylmalonate (IX), from 3,4,5-trimethoxybenzaldehyde (X) by successive reactions with sodium borohydride, thionyl chloride, and diethyl acetamidomalonate and its hydrolysis by 5 *N* HCl. The hydrolysis of the acetamidomalonate ester IX was reported to give the required amino acid VIII as its hydrochloride in 68% yield.

This procedure was repeated, and it was found that the acid hydrolysis of IX under identical conditions leads to a mixture of products. The hydrochloride, which separated on cooling the reaction mixture, was recrystallized from 5 *N* HCl (as reported in the previous procedure) to give needles, m.p. 241–242° dec. [lit. (6) m.p. about 242° dec.]. This crystalline product was found to be a mixture of four ninhydrin-positive compounds, designated A–D, by paper chromatography in Solvent System I, *n*-butanol–water–acetic acid (4:5:1). This finding is contrary to the report by Acheson *et al.* (6), who found their crystalline product to be a single-spot material by paper chromatography in Solvent Systems II, *n*-butanol–acetic acid–water (4:1:1), and III, isopropanol–ammonia–water (8:1:1). Four additional ninhydrin-positive components also were detected during paper chromatography of the mother liquor (the original reaction mixture after removal of the hydrochloride crystals) in Solvent System I. The chromatographic data on the eight products formed during the acid hydrolysis of IX are given in Table I.

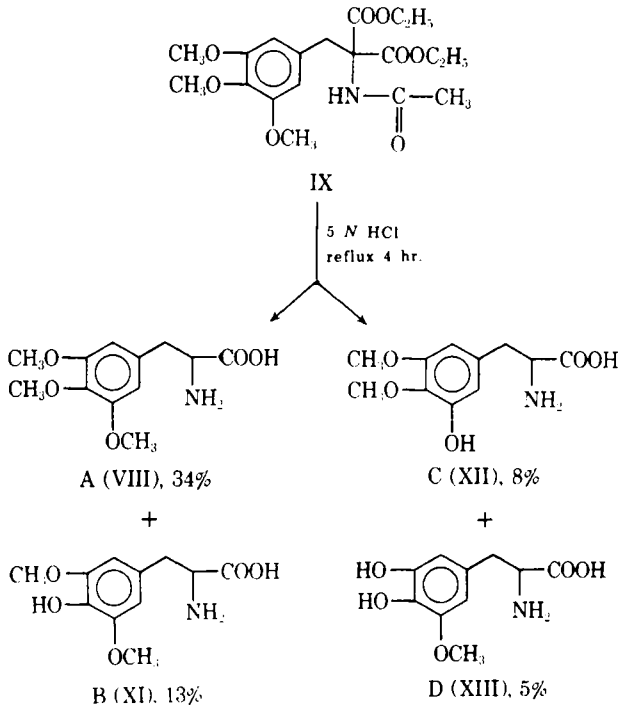
Isolation and Identification of Major Hydrolysis Products—Separation and isolation of the major hydrolysis products, A–D, were accomplished by preparative paper chromatography, and the following yields were obtained based on the isolated homogeneous products: A, 34%; B, 13%; C, 8%; and D, 5%. Identification of the isolated compounds (Scheme I) was carried out by NMR and mass spectral methods.

The major product, A, was obtained as fine needles, m.p. 243–244°, by recrystallization from aqueous hydrochloric acid. Based upon its NMR and mass spectra, Compound A was identified as the hydrochloride of the target amino acid, 3,4,5-trimethoxyphenylalanine (VIII). The mass spectrum of A showed the parent ion at *m/e* 255 and the base peak at *m/e* 181 corresponding to the trimethoxybenzyl ion (Scheme II).

The NMR spectrum (deuterium oxide solvent) of Compound A exhibited two singlets corresponding to the aromatic methoxyls at δ 3.87 (6H, methoxyls at C-3 and C-5) and 3.8 (3H, methoxyl at C-4), a singlet at δ 6.6 (2H) for the aromatic protons at C-2 and C-6, and multiplets at δ 3.2 (2H) and 3.7 (1H) p.p.m. assignable to the benzylic protons and the proton on the carbon bearing the carboxyl group, respectively.

The mass spectra of Compounds B and C indicated these two amino acids to be monodemethylated products of VIII. Both compounds showed parent ions at *m/e* 241 and the base peak at *m/e* 167 corresponding to the monohydroxydimethoxybenzyl ions (Scheme III).

Structural assignments to the monodemethylated amino acids, B (4-hydroxy-3,5-dimethoxyphenylalanine, XI) and C (3-hydroxy-4,5-dimethoxyphenylalanine, XII), were made on the basis of their NMR spectra in neutral and alkaline media. In deuterium oxide



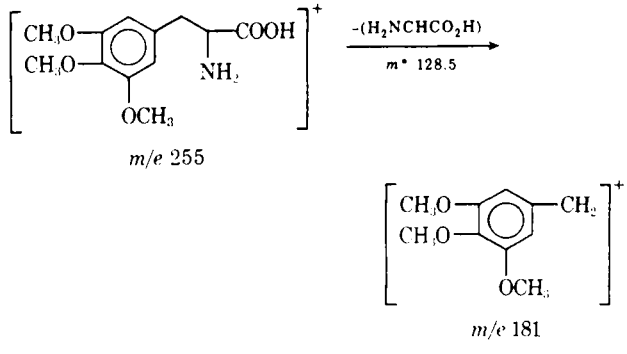
Scheme I

solvent, Compound B (XI) showed two singlets at δ 3.85 (6H, aromatic methoxyls at C-3 and C-5) and 6.65 (2H, aromatic protons at C-2 and C-6) p.p.m. Upon addition of sodium deuteroxide, the singlet corresponding to the aromatic protons at C-2 and C-6 in B (XI) shifted upfield by 0.11 Hz., which is consistent with their assignment to the *meta*-protons in relation to the phenolic hydroxyl at the C-4 position (7).

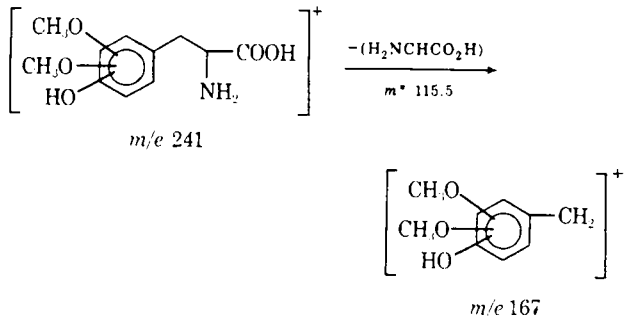
The NMR spectrum of C (XII) in deuterium oxide exhibited four singlets at δ 3.84 (3H, aromatic methoxyl at C-4), 3.86 (3H, aromatic methoxyl at C-3), 6.68 (1H, aromatic proton at C-2), and 6.71 (1H, aromatic proton at C-6) p.p.m. The two benzylic protons and the proton on the carbon bearing the carboxyl group in the spectra of acids B (XI) and C (XII) showed the expected multiplets, as in the case of trimethoxyphenylalanine (VIII).

Compound D was found to be a dihydroxymonomethoxy derivative of phenylalanine based on its mass spectrum, which showed the parent ion at *m/e* 227 and the base peak at *m/e* 153 arising from the loss of the fragment, H_2NCHCO_2H. Its NMR spectrum in deuterium oxide showed three singlets at δ 3.82 (3H, aromatic methoxyl at C-5), 6.69 (1H, aromatic proton at C-2), and 6.66 (1H, aromatic proton at C-6) p.p.m. and multiplets at δ 3.19 (2H, benzylic protons) and 3.71 (1H, proton on the carbon carrying carboxyl group) p.p.m. On the basis of these spectral characteristics, Compound D was identified as 3,4-dihydroxy-5-methoxyphenylalanine (XIII).

Chemical Method for Separation of VIII from Acid Hydrolysate—Since the separation of trimethoxyphenylalanine (VIII) from the phenolic amino acids, XI–XIII, formed during the acid hydrolysis



Scheme II



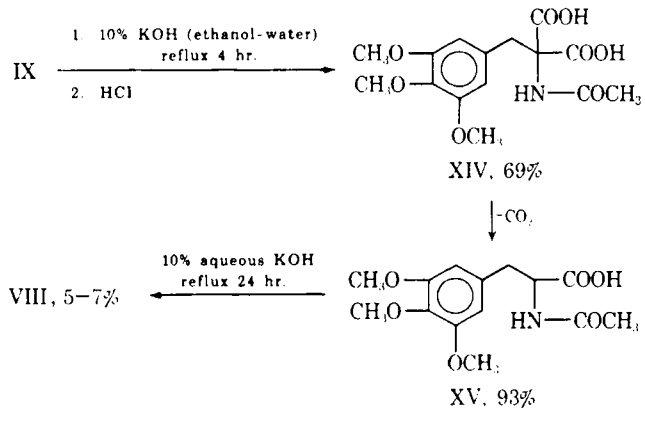
Scheme III

of the acetamidomalonate ester IX by the preparative paper chromatographic procedure was tedious, a chemical method for its isolation in larger quantities was developed.

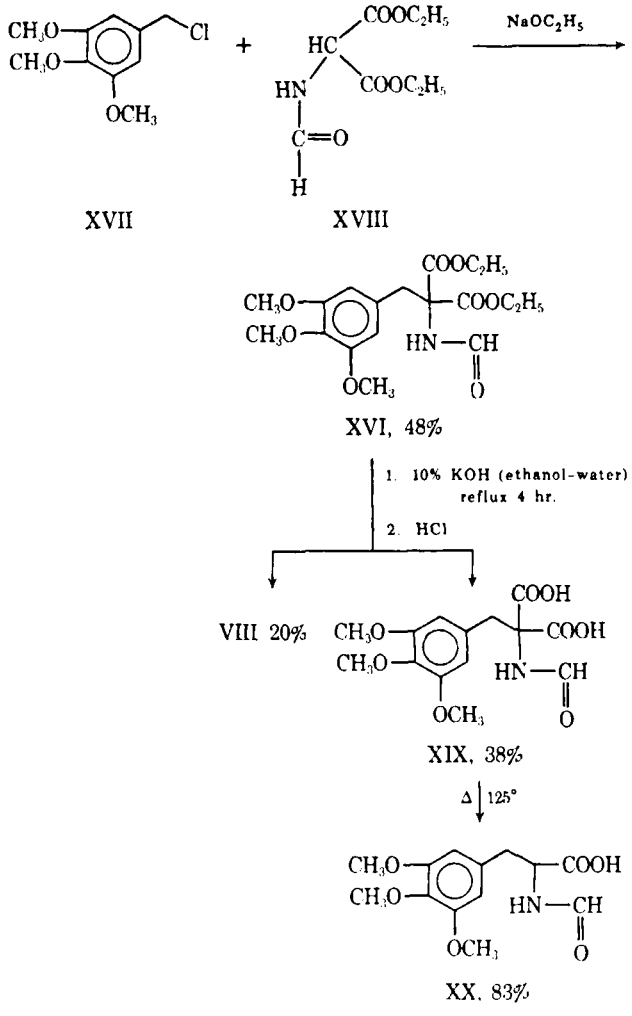
The hydrolysis mixture, consisting of VIII and accompanying phenolic amino acids, was refluxed with a methanol–sulfuric acid–ethylene dichloride mixture (4:0.4:6), and the resulting methyl esters were shaken with 10% NaOH and extracted with benzene. The benzene extract, upon removal of the solvent, gave exclusively the methyl ester of the nonphenolic amino acid, trimethoxyphenylalanine (30% yield), which was then hydrolyzed with 10% barium hydroxide to yield homogeneous trimethoxyphenylalanine (VIII) in 75% yield. The methyl esters of the phenolic amino acids, XI–XIII, were obtained from the aqueous sodium hydroxide layer by acidifying with carbon dioxide and extracting with chloroform.

Basic Hydrolysis of IX—To minimize the formation of phenolic amino acids during the hydrolysis of the acetamidomalonate ester (IX), the hydrolysis was carried out under basic conditions. Compound IX was refluxed for 4 hr. with 10% KOH in ethanol–water (1:1), which gave α-acetamido-α-3,4,5-trimethoxybenzylmalonic acid (XIV) in 69% yield. The dicarboxylic acid XIV readily decarboxylated when kept under vacuum, and the resulting *N*-acetyl-3,4,5-trimethoxyphenylalanine (XV, 93% yield) was again refluxed with 10% KOH. Hydrolysis of the *N*-acetyl derivative (XV) did not proceed smoothly, and only poor yields (5–7%) of trimethoxyphenylalanine (VIII) could be obtained. However, the hydrolysis product was found to consist solely of the required amino acid VIII and no phenolic amino acids were detected. Thus, hydrolysis of IX under basic conditions leads to a single product, VIII, but in low yields (Scheme IV).

Synthesis by Diethyl Formamidomalonate Method—Since hydrolysis of the acetamidomalonate ester IX under both acidic and basic conditions did not proceed satisfactorily, diethyl α-formamido-α-3,4,5-trimethoxybenzylmalonate (XVI) (Scheme V) was prepared and its hydrolysis was studied in an attempt to obtain better yields of the required trimethoxyphenylalanine (VIII). 3,4,5-Trimethoxybenzyl chloride (XVII) and diethyl formamidomalonate (XVIII) were condensed in the presence of sodium ethoxide to form the formamidomalonate ester, XVI (48% yield). Hydrolysis of the malonic ester XVI was carried out by refluxing for 4 hr. with 10% alcoholic KOH, which yielded the required VIII in 20% yield along with α-formamido-α-3,4,5-trimethoxybenzylmalonic acid (XIX, 38% yield). Compound VIII could be easily separated from



Scheme IV



Scheme V

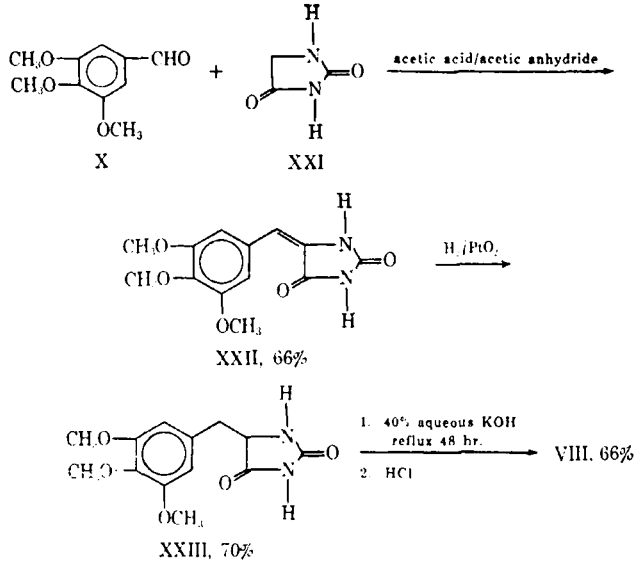
the reaction mixture by converting to its hydrochloride by acidification.

Decarboxylation of the malonic acid XIX by heating at 125° for 1 hr. gave *N*-formyltrimethoxyphenylalanine (XX) in 83% yield. However, as in the case of the *N*-acetyl derivative XV, basic hydrolysis of XX also gave low yields (5–7%) of 3,4,5-trimethoxyphenylalanine (VIII). During the basic hydrolysis of both acetyl and formyl derivatives, XV and XX, no phenolic products were encountered. This is in contrast to the hydrolysis under acidic conditions, which results in the formation of substantial quantities of phenolic amino acids.

Synthesis by Hydantoin Method—Although the condensation of 3,4,5-trimethoxybenzyl chloride (XVII) with both formamidomalonate and acetamidomalonate esters gave high yields of the corresponding acyl trimethoxybenzylmalonate esters, IX and XVI, their subsequent hydrolysis to the requisite 3,4,5-trimethoxyphenylalanine (VIII) proceeded in low yields. Both the formyl and acetyl derivatives of trimethoxyphenylalanine, XX and XV, formed during the synthetic procedures were quite resistant to hydrolytic cleavage.

To circumvent the formation of acyl derivatives of trimethoxyphenylalanine during the synthetic procedure, an alternative method involving a hydantoin derivative was tried (Scheme VI). 3,4,5-Trimethoxybenzaldehyde (X) was condensed with hydantoin (XXI) by refluxing for 24 hr. in glacial acetic acid–acetic anhydride, and the resulting 5-(3,4,5-trimethoxybenzylidine)hydantoin (XXII, 66% yield) was hydrogenated over platinum oxide to yield 5-(3,4,5-trimethoxybenzyl)hydantoin (XXIII, 70% yield). Basic hydrolysis of the hydantoin derivative XXIII gave high yields (66%) of the target amino acid, 3,4,5-trimethoxyphenylalanine (VIII).

GC–Mass Spectrometry of Peyote Amino Acid Fraction—During these synthetic studies, several trioxygenated phenylalanine derivatives (XI–XIII) were obtained in addition to 3,4,5-trimethoxyphen-



Scheme VI

ylalanine (VIII), the analog corresponding to mescaline. By using these as the reference compounds, the amino acid fraction of the peyote cactus was examined for their possible natural occurrence by the GC–mass spectrometric procedure described earlier (1–3). No evidence was obtained for the presence of trioxygenated analogs of phenylalanine in the peyote. This observation may be significant, since it may be taken as an indication that the carboxyl group does not survive beyond the stage of dihydroxyphenylalanine in the biosynthetic sequence leading to the trioxygenated alkaloids of peyote.

EXPERIMENTAL[1]

Compounds were visualized on paper chromatograms with the aid of ninhydrin and diazo spray reagents. The following compounds were purchased from a commercial source[2]: 3,4,5-trimethoxybenzaldehyde, diethyl formamidomalonate, diethyl acetamidomalonate, and hydantoin.

Diethyl α-Acetamido-α-3,4,5-trimethoxybenzylmalonate (IX)—Compound IX was synthesized according to the procedure of Acheson *et al.* (6). Diethyl acetamidomalonate (11 g.) was dissolved in a solution of sodium ethoxide (sodium, 1.18 g. in absolute alcohol, 75 ml.) by warming. To this stirred solution was added 3,4,5-trimethoxybenzyl chloride (10 g.) in five portions. The reaction mixture was refluxed for 6 hr., cooled, and filtered. The filtrate was concentrated to approximately half the volume, and water (50 ml.) was added. Extraction with ether (2 × 50 ml.) and evaporation of the dried (sodium sulfate) ether extract gave the ester IX (6.6 g.), which crystallized from benzene–petroleum ether as flakes, m.p. 116–117° [lit. (6) m.p. 116°].

Acid Hydrolysis of Acetamidomalonate Ester IX—The acetamidomalonate ester IX (907 mg.) was refluxed with 5 *N* HCl (30 ml.) for 4 hr. Upon cooling the reaction mixture, fine needles separated which were collected by filtration (392 mg.) and recrystallized from 5 *N* HCl to give needles, m.p. 241–242° dec. Acheson *et al.* (6) reported a melting point of about 242° dec. for their crystalline product, which they claimed to be 3,4,5-trimethoxyphenylalanine (VIII) hydrochloride.

Paper chromatographic data on this crystalline product and the mother liquor are given in Table I. The crystalline product consisted of four ninhydrin-positive components, A–D, while four additional compounds, E–H, were detected in the mother liquor.

[1] Melting points were taken on a Kofler micro-hot-stage and are corrected. NMR chemical shifts are given in parts per million downfield from tetramethylsilane as an internal standard. Mass spectra were recorded at 70-ev, electron beam voltage. Spectra were recorded on Varian XL-100 NMR and LKB-9000 GC–mass spectrometers. Paper chromatography was performed on Whatman No. 1 paper.

[2] Aldrich Chemical Co.

Table I—Paper Chromatography of Acid Hydrolysis Products of Diethyl α-Acetamido-α-3,4,5-trimethoxybenzylmalonate (IX)

Sample Chromatographed	Compounds Detected[a]	R_f Value in Solvent System *n*-Butanol–Water–Acetic Acid (4:5:1)	Yield[b], %
Hydrochloride crystals	A	0.56	34
	B	0.34	13
	C	0.43	8
	D	0.40	5
Mother liquor	E	0.81	Trace
	F	0.67	Trace
	G	0.22	Trace
	H	0.12	Trace

[a] See Scheme I for identification of Compounds A–D. [b] Yields reported are based on homogeneous products isolated by preparative paper chromatography (see *Experimental* for details).

A portion of this crystalline product (300 mg.) was subjected to preparative paper chromatography in Solvent System I and the following homogeneous products were isolated: A (110 mg.), B (42 mg.), C (26 mg.), and D (16 mg.). Identification of the isolated compounds, A–D, was carried out with the aid of their NMR and mass spectra (see *Results and Discussion* and Scheme I).

Chemical Method for Isolation of 3,4,5-Trimethoxyphenylalanine (VIII) from Acid Hydrolysate—The crystalline product (398 mg.) obtained during the acid hydrolysis of the acetamidomalonate ester IX was refluxed with ethylene dichloride (0.6 ml.) and concentrated sulfuric acid (75 mg.) in methanol (0.4 ml.) for 16 hr. The reaction mixture was cooled, the solvent was removed *in vacuo*, and water (2 ml.) was added. The aqueous solution was basified with 10% NaOH and extracted with benzene (3 × 5 ml.). Evaporation of the dried (sodium sulfate) benzene extract yielded the methyl ester of 3,4,5-trimethoxyphenylalanine (120 mg.). This methyl ester was hydrolyzed by refluxing with 10% $Ba(OH)_2$ for 24 hr., which yielded 3,4,5-trimethoxyphenylalanine (VIII) in 75% yield. From the aqueous sodium hydroxide solution, esters of the phenolic amino acids XI–XIII were obtained by acidification with carbon dioxide and extraction with chloroform.

Basic Hydrolysis of Acetamidomalonate Ester IX—Compound IX (1.02 g.) was dissolved in 10 ml. of ethanol–water (1:1) and refluxed with 10% KOH (20 ml.) for 4 hr. Ethanol was then removed *in vacuo* from the reaction mixture and acidified with concentrated hydrochloric acid. The crystalline precipitate formed was filtered, washed with cold water, and dried (0.69 g.) to give acetamido-3,4,5-trimethoxybenzylmalonic acid (XIV), m.p. 135–137°. Upon keeping the malonic acid XIV under vacuum for 2 days, decarboxylation occurred and gave 0.64 g. of *N*-acetyl-3,4,5-trimethoxyphenylalanine (XV), m.p. 190–191.5°.

Anal.—Calc. for $C_{14}H_{19}NO_6$: C, 56.56; H, 6.44; N, 4.71. Found: C, 56.38; H, 6.29; N, 4.67.

The *N*-acetyl derivative XV (0.102 g.) was refluxed with 10% KOH (25 ml.) for 20 hr. The reaction mixture was cooled and passed through a column of cation-exchange resin[3] (20 ml.). An additional 300 ml. of water was passed through the column, and the combined eluate was evaporated to dryness. The resulting residue was taken up in dilute hydrochloric acid (5 ml.) which, upon cooling, gave 7.3 mg. of 3,4,5-trimethoxyphenylalanine (VIII) as its hydrochloride, m.p. 243–244°.

Diethyl α-Formamido-α-3,4,5-trimethoxybenzylmalonate (XVI)—Diethyl formamidomalonate (XVIII) (2.02 g.) and 3,4,5-trimethoxybenzyl chloride (XVII) (2.15 g.) were added to a solution of sodium ethoxide (250 mg. of sodium dissolved in 12 ml. of absolute alcohol) and refluxed for 14 hr. The reaction mixture was filtered, and the filtrate was evaporated to dryness. The residue was suspended in water (50 ml.) and extracted with ether (3 × 50 ml.). Upon evaporation, the dried (sodium sulfate) ether extract gave 1.82 g. of crude product, m.p. 117–118°. Crystallization of this product from benzene–petroleum ether gave 1.68 g. of diethyl α-formamido-α-3,4,5-trimethoxybenzylmalonate (XVI), m.p. 120–121°.

[3] Amberlite, IRC 50 (H), Rohm & Haas Co.

Anal.—Calc. for $C_{18}H_{25}NO_8$: C, 56.39; H, 6.57; N, 3.65. Found: C, 56.45; H, 6.64; N, 3.55.

Basic Hydrolysis of Formamidomalonate Ester XVI—Compound XVI (0.6 g.) was refluxed with a mixture of 10% KOH (6 ml.) and ethanol–water (1:1, 14 ml.) for 4 hr. The reaction mixture was concentrated under argon atmosphere to 3 ml., cooled, and acidified with 18% HCl (4 ml.). Upon cooling the acidic solution, 192 mg. of formamido-3,4,5-trimethoxybenzylmalonic acid (XIX) separated out, m.p. 126–127°.

The mother liquor was freeze dried and the residue was shaken with hot methanol (3 × 15 ml.). The methanol extract was evaporated to dryness, and the residue was dissolved in water (5 ml.) and extracted with chloroform (5 × 10 ml.). Upon removal of the solvent, the chloroform extract gave 55 mg. of the malonic acid XIX. The aqueous layer was evaporated to dryness and the residue (350 mg.) was extracted with methanol (3 × 5 ml.). The methanol extract was left in the refrigerator overnight and deposited needles of 3,4,5-trimethoxyphenylalanine (VIII) as the hydrochloride (125 mg.), m.p. 243–244°.

Decarboxylation of the malonic acid XIX (55 mg.) was carried out by heating at 125° for 1 hr., which gave 40 mg. of *N*-formyl-3,4,5-trimethoxyphenylalanine (XX), m.p. 154–155°.

Anal.—Calc. for $C_{13}H_{17}NO_6$: C, 55.12; H, 6.05; N, 4.95. Found: C, 55.47; H, 6.22; N, 4.49.

Basic hydrolysis of the *N*-formyl derivative XX (30 mg.) was carried out as in the case of the *N*-acetyl derivative XV, which gave 2.2 mg. of 3,4,5-trimethoxyphenylalanine (VIII) as the hydrochloride, m.p. 243–244°.

5-(3,4,5-Trimethoxybenzylidine)hydantoin (XXII)—Hydantoin (5 g.) and 3,4,5-trimethoxybenzaldehyde (9.8 g.) were dissolved in a mixture of glacial acetic acid (15 ml.) and acetic anhydride (2 ml.) and refluxed for 24 hr. The reaction mixture was evaporated to dryness *in vacuo*, and the residue was taken up in cold water (500 ml.). Upon vigorous shaking, a yellow crystalline precipitate separated which was collected on a funnel by filtration and washed with cold water. The filtrate was extracted with chloroform (10 × 100 ml.) and the chloroform extract was evaporated to dryness, yielding additional quantities of the yellow crystalline product. The two yellow crystalline products were combined and recrystallized from ethanol–chloroform to yield 8.96 g. of 5-(3,4,5-trimethoxybenzylidine)hydantoin (XXII) as needles, m.p. 268–269°.

Anal.—Calc. for $C_{13}H_{14}N_2O_5$: C, 56.11; H, 5.07; N, 10.07. Found: C, 56.04; H, 5.06; N, 10.15.

5-(3,4,5-Trimethoxybenzyl)hydantoin (XXIII)—A suspension of XXII (2.61 g.) in methanol (40 ml.) was acidified with a few drops of dilute hydrochloric acid and hydrogenated in the presence of platinum oxide (200 mg.) at 30 p.s.i. pressure for 18 hr. The resulting colorless solution was filtered and evaporated to dryness. Crystallization of the resulting residue from methanol–benzene afforded 1.81 g. of white microcrystals of XXIII, m.p. 180–181°.

Anal.—Calc. for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.75; N, 10.00. Found: C, 56.01; H, 5.87; N, 10.18.

3,4,5-Trimethoxyphenylalanine (VIII)—A solution of trimethoxybenzylhydantoin (XXIII, 280 mg.) in ethylene glycol monoethyl ether (5.5 ml.) was refluxed with potassium hydroxide (0.8 g. in 2 ml. of water) for 48 hr. The reaction mixture was evaporated to dryness, and the residue was suspended in water (5 ml.) and acidified with 10% HCl. The aqueous acidic solution was then evaporated to dryness, and the resulting residue was extracted with hot absolute alcohol (4 × 20 ml.). During the concentration of the alcohol extract, needle-shaped crystals appeared which were collected and recrystallized from absolute alcohol to give 193 mg. of 3,4,5-trimethoxyphenylalanine (VIII) as the hydrochloride as needles, m.p. 243–244°.

Anal.—Calc. for $C_{12}H_{17}NO_5 \cdot HCl$: C, 49.41; H, 6.17; N, 4.80. Found: C, 49.18; H, 6.33; N, 4.84.

SUMMARY AND CONCLUSIONS

1. Acid hydrolysis of the acetamidomalonate ester (IX) in the procedure of Acheson *et al.* (6) for the synthesis of 3,4,5-trimethoxyphenylalanine was shown to lead to a mixture of products. With the aid of preparative paper chromatography, the major hydrolysis products were isolated and identified by NMR and mass spectrometry as 3,4,5-trimethoxyphenylalanine (VIII, 34%), 4-hydroxy-3,5-dimethoxyphenylalanine (XI, 13%), 3-hydroxy-4,5-dimethoxyphenylalanine (XII, 8%), and 3,4-dihydroxy-5-methoxyphenyl-

alanine (XIII, 5%). A chemical method was developed for the separation of the amino acid VIII from the phenolic products present in the hydrolysis mixture.

2. Under basic conditions, hydrolysis of the acetamidomalonate ester IX gave low yields (5–7%) of the required amino acid VIII. However, no phenolic side-products were formed under this condition.

3. Synthesis of the formamidomalonate ester XVI and its hydrolysis under basic conditions were carried out in an effort to realize higher yields of trimethoxyphenylalanine (VIII). The hydrolysis of the ester XVI also gave unsatisfactory yields of the target amino acid VIII.

4. An efficient alternative synthesis of trimethoxyphenylalanine (VIII) was achieved which involves the formation of the hydantoin derivative XXIII and its hydrolysis under basic conditions (66% yield).

5. GC–mass spectrometric examination of the peyote amino acid fraction gave no evidence for the presence of trioxygenated phenylalanine analogs, VIII and XI–XIII. This observation suggests that the carboxyl group may not survive beyond the stage of dopa during the biosynthesis of the trioxygenated peyote alkaloids.

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Enhanced Absorption of Digitoxin from Orally Administered Digitoxin–Polyvinylpyrrolidone Coprecipitates

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Abstract □ The general applicability of the polyvinylpyrrolidone coprecipitation technique as a method for enhancing the GI absorption of orally administered hydrophobic drugs was explored with the cardiac glycoside digitoxin. The relative absorption characteristics of digitoxin alone and as a 1:9 (w/w) physical mixture and coprecipitate with polyvinylpyrrolidone were determined indirectly by measuring their oral LD_{50} values in rats. The *in vivo* data obtained provided evidence that digitoxin was absorbed from the coprecipitate at a significantly faster rate and was present in the body at a much higher level than when equivalent doses of either the drug alone or as a physical mixture with polyvinylpyrrolidone were orally administered. For example, one must orally administer approximately 11 times as much pure drug to reach the same amount of drug in the body as that attained following the administration of the drug as a coprecipitate. A correlation was found between the *in vitro* dissolution rates of these test systems at 37° and their *in vivo* toxicities.

Keyphrases □ Digitoxin absorption—enhanced using orally administered coprecipitate with polyvinylpyrrolidone, compared to separate drug and physical mixture □ Polyvinylpyrrolidone coprecipitate with digitoxin—enhanced *in vivo* absorption, compared to separate drug and physical mixture □ Coprecipitates, digitoxin–polyvinylpyrrolidone—enhanced *in vivo* drug absorption □ Drug absorption, digitoxin—enhanced using orally administered polyvinylpyrrolidone coprecipitate

Among the techniques that can potentially enhance the dissolution rate and, hence, the rate and/or extent of absorption of hydrophobic drugs is the formation of coprecipitates with pharmacologically inert, polymeric materials such as polyvinylpyrrolidone. This physicochemical drug modification offers the advantage of possibly enabling one to administer the drug orally in a form from which it is most available for GI absorption. Although several investigations (1–6) have demonstrated, *in vitro*, that the solubility and/or dissolution rates of drugs can be increased in this manner, little information is available in the literature related to the *in vivo* absorption pattern of drugs orally administered as polyvinylpyrrolidone coprecipitates. Recently, however, it was demonstrated (6) that both the rate and extent of absorption of the water-insoluble drug reserpine could be markedly enhanced when orally administered to rats in the form of a 1:5 (w/w) coprecipitate with polyvinylpyrrolidone.

The purpose of the present investigation was to ascertain, *in vivo*, the general applicability of the polyvinylpyrrolidone coprecipitation technique. To accomplish this aim, the absorption characteristics of digitoxin, a 1:9 (w/w) digitoxin–polyvinylpyrrolidone physical mix-