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BROMINATED PHENYLALANINES.

M.L.Anhoury, P.Crooy, R.De Neys and J.Eliaers.
Research and Development, R.I.T. S.A., Genval, Belgium.

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β-(3-Bromo-4-hydroxy-5-methoxy-phenyl)alanine (Ie) exerts a long-acting antihypertensive effect in rats and dogs : its synthesis and its optical resolution have already appeared in the literature.[1] Ten new mono- and polybrominated phenylalanines with hydroxy- and/or methoxy-groups are here described. These unnatural amino-acids were synthesized by direct bromination, or via the acetamidomalonic esters (V), or via the azlactones (VI) and the corresponding phenylpyruvic acids (VIII).

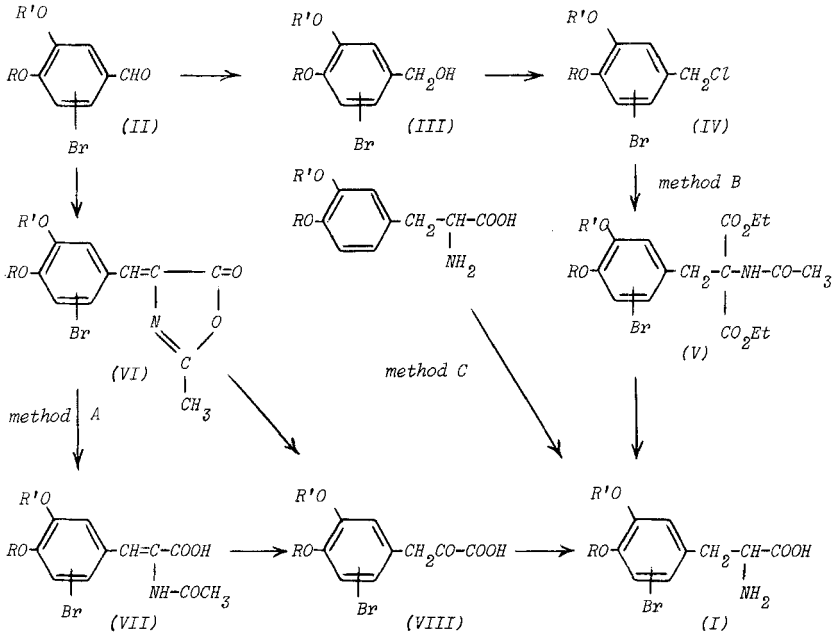
INTRODUCTION.

In a preceding paper,[1] we described the synthesis, the separation and the absolute configuration of both optical isomers of DL-β (3-bromo-4-hydroxy-5-methoxyphenyl)alanine (Ie) (RIT 1412), a potential long acting antihypertensive agent in Grollman rats [2] when injected by the s.c. route.

The purpose of this paper is to describe the synthesis and the physical properties of some other brominated phenylalanines, substituted by a RO-group in the benzene nucleus at the meta and para positions, R being a methyl or a hydrogen residue.

All these substituted phenylalanines were obtained by one of the three methods mentioned in scheme I.

Scheme I.



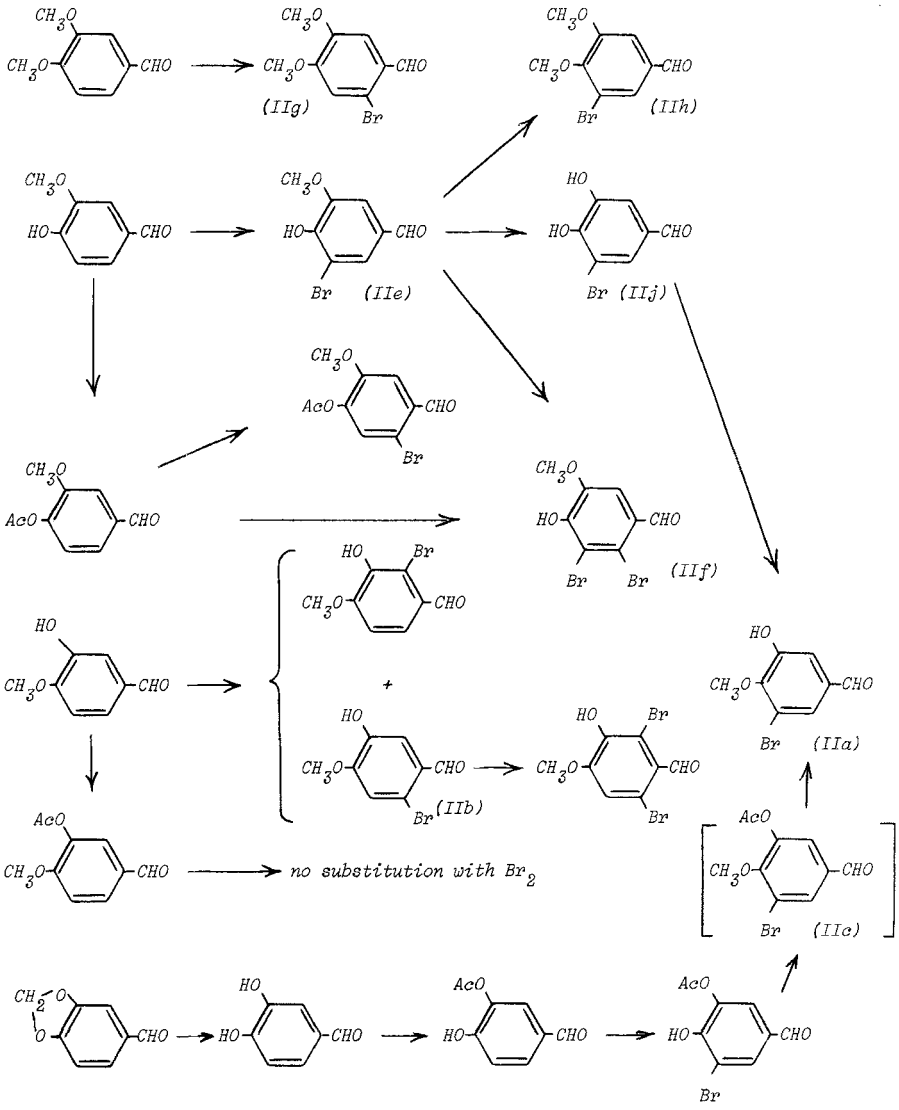
a	3-Br	4-OCH_3	5-OH	*g*	2-Br	4-OCH_3	5-OCH_3
b	2-Br	4-OCH_3	5-OH	*h*	3-Br	4-OCH_3	5-OCH_3
c	3-Br	4-OCH_3	5-OAc	*i*	2-Br	4-OH	5-OH
d	2-Br	4-OH	5-OCH_3	*j*	3-Br	4-OH	5-OH
e	3-Br	4-OH	5-OCH_3	*k*	2,3-diBr	4-OH	5-OH
f	2,3-diBr	4-OH	5-OCH_3	*m*	2,6-diBr	4-OH	3-OH
				n	2,3,6-triBr	4-OH	5-OH

If the brominated phenylalanine (I) can not be obtained by direct halogenation of the adequate substituted phenylalanine (method C), one has the choice between the hydrolysis of the acetamidomalonic ester (V) in a hydrochloric acid - acetic acid medium (method B) and the borohydride reduction of a substituted phenylpyruvic acid (VIII) in concentrated aqueous ammonia (method A). These phenylpyruvic acids may be obtained directly [1] *from the corresponding azlactones (VI) or through the* α*-acetamidocinnamic acids (VII).*

A. SYNTHESIS OF STARTING SUBSTITUTED BENZALDEHYDES.

The common starting material of each of these amino acids is an adequately brominated 3,4-substituted benzaldehyde (II) as shown in scheme II.

Scheme II.



Veratraldehyde is halogenated in position 6 to yield (IIg),[3] *while vanillin gives 5-bromovanillin (IIe),*[3,4,5,6] *which can then be converted to 5-bromoveratraldehyde (IIh) by means of dimethylsulfate,*[3,5,7] *to 5-bromoprotocatechualdehyde (IIj) using anhydrous aluminium trichloride and pyridine in methylene chloride* [8] *and to 5,6-dibromovanillin (IIf) by means of a second molecule of bromine in hot acetic acid.*[9] *Vanillin can be easily acetylated to O-acetylvanillin.*[10] *In this molecule, the bromine atom enters into position 6.*[11] *The action of two molecules of bromine on O-acetylvanillin gives raise to 5,6-dibromovanillin (IIf)*[9] *after deacetylation. Bromination of isovanillin occurs in position 2 or 6, the ratio of the two isomers depending on the experimental conditions.*[12] *6-Bromoisovanillin (IIb) is halogenated to 2,6-dibromoisovanillin according to Hazlet and Brotherton.*[12] *Unfortunately, O-acetylisovanillin* [13] *does not brominate at all in the usual conditions.*[12,13] *The only product of the reaction is some 5-bromoguaiacol. The only synthesis of 5-bromoisovanillin (IIa), described in 1962, is due to Hazlet and Brotherton.*[12] *It is a four step synthesis, starting from piperonal, the total yield from this material to 5-bromoisovanillin being 4% .*

In this paper, we propose a three step synthesis, starting from vanillin. After a quantitative bromination of vanillin in position 5, the obtained product (IIe) is demethylated to give 5-bromoprotocatechualdehyde (IIj), which is then selectively methylated in position 4 to give 5-bromoisovanillin (IIa) by methyl iodide and sodium bicarbonate in acetone or by diazomethane in dioxane-ether. The total yield of this synthesis, starting from vanillin is 54% . The structure of 5-bromoisovanillin (IIa) was confirmed by chemical transformation to 5-bromoisovanillic acid.

B. SYNTHESIS OF α-AMINO ACIDS.

a) Method A : β-(3-bromo-5-hydroxy-4-methoxyphenyl)alanine (Ia) was synthesized via the α-keto-acid obtained in one or two steps from the azlactone.

b) Method B : most of the benzyl alcohol derivatives (III) used are already described.[3,14] *These alcohols were synthesized in our laboratory by reduction of the corresponding aldehyde (I M) by means of sodium borohydride (0.6 M), the solvent being dioxane or dimethylformamide.*

The substituted benzyl chlorides (IV) were obtained by treatment of the corresponding benzyl alcohols (III) by a stream of hydrogen chloride in dry benzene. The 3,4-dimethoxybenzyl chlorides with a bromine atom in position 5 and 6 are known.[3,15]

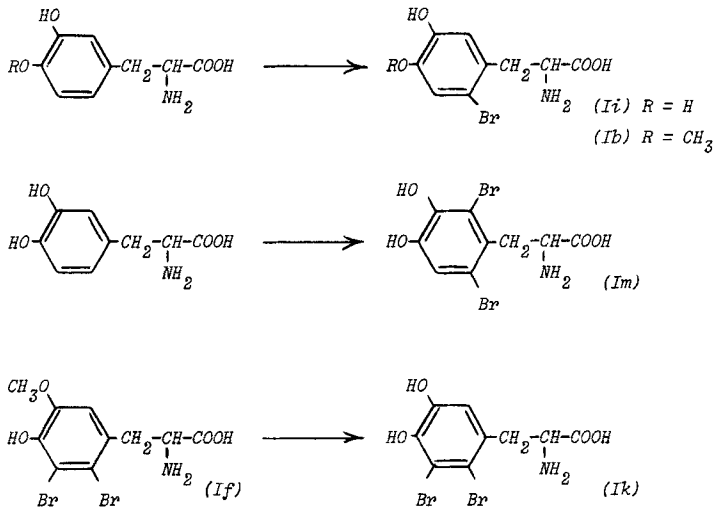
These benzyl chlorides (IV) were condensed with diethyl acetamidomalonate in dimethylformamide, the condensing agent being sodium hydride.

Diethyl(3-bromo-4,5-dihydroxy)benzylacetamidomalonate (Vj) was prepared by demethylation of the 5-methoxy analog (Ve), using aluminium trichloride and pyridine in methylene chloride as a solvent. Hydrolysis of these malonic esters in a hydrochloric acid - acetic acid medium gave rise to the desired amino-acids.

c) Method C : bromination of β-(3-hydroxy-4-methoxyphenyl)alanine in acetic acid affords β-(2-bromo-5-hydroxy-4-methoxyphenyl)alanine (Ib) (scheme III).

This was proved by nuclear magnetic resonance and by degradation of the molecule.

Scheme III.



Dopa also gives 6-bromo-dopa (Ii) in the same experimental conditions.[16]

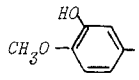
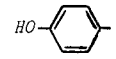
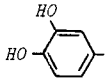
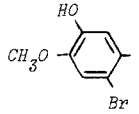
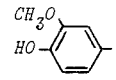
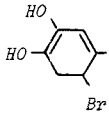
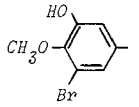
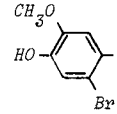
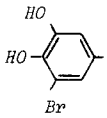
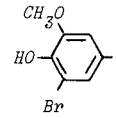
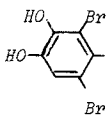
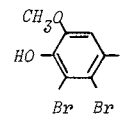
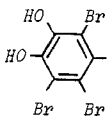
J.RUTSCHMANN and E.SCHREIER described the product of reaction of dopa with two molecules of bromine as X-6 dibromo-DL-dopa.[16] *We have chromatographic evidence that this reaction gives 2,6-dibromo-dopa (Im). Indeed, this amino-acid has a different Rf from 5,6-dibromodopa (Ik), obtained by demethylation of β-(2,3-dibromo-4-hydroxy-5-methoxyphenyl)alanine (If). 2,3,6-Tribromodopa (In) is obtained directly from dopa.*[17]

For each phenolic aminoacid, the pK_a value of the phenolic group has been determined by ultraviolet spectrophotometry.[18]

As shown in Table 1, the position and the number of Br atoms influences the acidity of the phenolic group. The pK_a values were correlated to the antihypertensive effect.

Table 1.

Phenolic pK_a values of substituted phenylalanines

	10.4		10.1		8.2
	9.1		9.7		8.0
	8.4		8.8		7.6
			7.9		7.2
			7.3		5.8

EXPERIMENTAL.

- *The UV spectra were carried out in water at the concentration of 3.75 10^{-4} M/l.*
- *Melting points are uncorrected and determined in capillary tubes.*
- *The ^{1}H-nmr spectra were recorded on a Perkin-Elmer R-12 (60 Mc.) using acetonitrile or sodium 2,2-dimethyl-2-silapentanesulfonate as an internal reference ; the solvent was deuterium oxide or a mixture of deuterium oxide with hexadeuterodimethylsulfoxide (DMSO-d6).*
- *R_F values refer to paper chromatography analysis carried out on Whatman 3MM with n-butanol saturated with 3N-hydrochloric acid as solvent and ninhydrin as spray reagent.*
- *The elemental analyses were performed on a Hewlett Packard 185 CHN Analyzer.*
- *The purity of the amino-acids was determinated by potentiometric titration of the ionisable groups.*
- *pK_a values of the phenolic group were determined by UV spectroscopy using buffer solutions between pH 2 and 11, with a final concentration in amino-acid of about 2.5-3.5 10^{-4} M/l. The buffer solutions were sodium orthophosphate/citric acid (pH 2.2 to 8), orthoboric acid/sodium hydroxide (pH 7.8 to 10) and glycine/sodium hydroxide (pH 8.5 to 12.8).*

All the physical data of the α-amino-acids are recorded in table III & IV.

A. SYNTHESIS OF SUBSTITUTED BENZALDEHYDES (II).

O-Acetylvanillin,[10] *O-Acetyl-6-bromovanillin,*[11] *5-Bromovanillin (IIe),*[1,3-6] *5,6-Dibromovanillin (IIf),*[9] *5-Bromoveratraldehyde (IIh),*[3,5,7] *6-Bromoveratraldehyde (IIg),*[3] *5-Bromoprotocatechualdehyde (IIj)*[8] *are synthesized according to the literature.*

5-Bromoisovanillin (IIa). - (a) 5-Bromoprotocatechualdehyde (IIj)[8] *(108.5 g.), sodium bicarbonate (43.3 g.) and methyliodide (100 ml.) in dry acetone (700 ml.) are refluxed for 20 hr. The mixture is then concentrated under reduced pressure.*

The residue is taken up with chloroform, treated with a stream of dry hydrogen chloride. The precipitate formed is discarded and the obtained solution after purification yields 5-bromoisovanillin (IIa) (63 g., 54.5%) m.p. 116.5-117.5°, purity by potentiometric titration, 100.1% (OH).

(b) 5-Bromoprotocatechualdehyde (IIj) (2.17 g.) in dioxane (200 ml.) is treated dropwise by an ethereal solution (17.6 ml.) of diazomethane (2.39 g./100 ml.). After the end of the nitrogen evolution, the solution is concentrated to dryness under vacuo. The residue is taken up with chloroform and treated as above. The yield, m.p. and purity are the same as in (a).

Proof of structure (IIa) : A suspension of silveroxide (2.3 g.) in aqueous sodium hydroxide solution (50 ml.), warmed to 70° is treated with the above 5-bromoisovanillin (4.6 g.). The reaction mixture is stirred for 2 hr. at room temperature and filtered over charcoal. The filtrate is acidified with a stream of sulfur dioxide. The precipitate is filtered, washed with water and dried under vacuo to yield 5-bromoisovanillic acid (2.2 g., 44.5%), m.p. 202.5-204°. Purity by potentiometric titration, 95.2% (CO_2H).

This acid (0.5 g.) is hydrogenated during 4 hr. at 44 psi in the presence of palladium 5% on charcoal (0.6 g.), sodium hydroxide (0.1 g.) and water (100 ml.). The catalyst is removed and isovanillic acid (0.2 g.) is obtained by acidification of the filtrate at pH 2 - m.p. 258-259.5°. There is no depression of the m.p. when this acid is mixed with an authentic sample of isovanillic acid.

By thin layer chromatography, the following Rf values are obtained on silicagel, using the mixture benzene-dioxane-acetic acid (90:25:4) as solvent and ferric chloride as detecting agent : isovanillic acid Rf 0.44, 5-bromoisovanillic acid Rf 0.39, hydrogenated 5-bromoisovanillic acid Rf 0.44.

B. SYNTHESES OF SUBSTITUTED PHENYLALANINES (I).

a) Scheme A.

4-(5-Acetoxy-3-bromo-4-methoxybenzylidene)-2-methyl-5-oxazolone (VIc). - 5-Bromoisovanillin (IIa) (32.4 g.), acetylglycine (16.4 g.), anhydrous sodium acetate (22.9 g.) and acetic anhydride (27 ml.) are heated for 2 hr. at 110°. This mixture is then poured in water (800 ml.), yielding the azlactone (31 g., 62.5%), m.p. 210-211° after one crystallization in acetic acid.

α -Acetamido-3-bromo-5-hydroxy-4-methoxycinnamic Acid (VIIa). - The above azlactone (20 g.) is dissolved in 2 N-sodium hydroxide (250 ml.) and stirred at 70° for 1 hr. The solution is then brought to a pH of 1.5 with 6 N-hydrochloric acid and heated for 1 hr. at 70°. The substituted cinnamic acid (VIIa) crystallizes (16 g., 85.8%), m.p. 218-219°. Purity by potentiometric titration, 99% (CO_2H); 100% (OH).

- By thin layer chromatography on silicagel, one spot, of Rf 0.25, can be detected by ferric chloride reagent. Solvent : benzene-methanol-acetic acid (45:8:4).

3-Bromo-5-hydroxy-4-methoxyphenylpyruvic Acid (VIIIa). - The above cinnamic acid (12 g.) is refluxed at 95° during 5 hr. in acetic acid (30 ml.) and concentrated hydrochloric acid (70 ml.). The hot solution is filtered on charcoal and cooled. By cooling, the substituted phenylpyruvic acid (VIIIa) crystallizes (8.6 g., 82%), m.p. 203-205°. Purity by potentiometric titration, 98% (CO_2H) ; 99.5% (OH).

- By thin layer chromatography on silicagel, one spot, of Rf 0.23, can be detected by the ferric chloride reagent. Solvent : benzene-methanol-acetic acid (45:8:4).

β -(3-Bromo-5-hydroxy-4-methoxyphenyl) alanine .HCl (Ia). - The substituted phenylpyruvic acid (VIIIa) (5 g.) is dissolved in 28% ammonium hydroxide (50 ml.) and stirred for 15 minutes. Sodium borohydride (0.5 g.) is then added to the obtained solution which is stirred for 4 hr.; then the pH is brought to 1.5 with 6 N-hydrochloric acid.(Ia)crystallizes in butanol (4.0 g., 71%).

b) Scheme B.

1. Synthesis of substituted benzyl alcohols III.

2,3-Dibromo-4-hydroxy-5-methoxybenzyl Alcohol (IIIf),[14] *2-Bromo-4,5-dimethoxybenzyl Alcohol (IIIg),*[3] *3-Bromo-4,5-dimethoxybenzyl Alcohol (IIIh),*[3] *3-Bromo-4-hydroxy-5-methoxybenzyl Alcohol (IIIe)*[1] *are synthesized according to the literature by reduction of the corresponding aldehydes with sodium borohydride.*

2-Bromo-4-hydroxy-5-methoxybenzyl Alcohol (IIId). - O-Acetyl-6-bromo vanillin (175 g.),[11] *10% sodium hydroxide (400 ml.), water (1,500 ml.) and sodium borohydride (15 g.) are stirred 1 day at 25°. The benzylalcohol is precipitated by 6 N-hydrochlorid acid at 0°, filtered, washed with cool water and dried to yield (IIId) (104.3 g., 70%) m.p. 152-155°.*

2. Syntheses of substituted benzyl chlorides (IV).

2-Bromo-4,5-dimethoxybenzyl Chloride (IVg)[15] *and 3-Bromo-4-hydroxy-5-methoxybenzyl Chloride (IVe) are synthesized according to the literature.*

Starting from the substituted benzyl alcohols (IIId, IIIf, IIIh) and using the method of synthesis described in 1, the following benzyl chlorides are obtained :

2-Bromo-4-hydroxy-5-methoxybenzyl Chloride (IVd) : yield 94.6%, purity by potentiometric titration, 95.0% (-OH).

2,3-Dibromo-4-hydroxy-5-methoxybenzyl Chloride (IVf) : yield 98.7%, m.p. 151-154°, purity by potentiometric titration, 96.2% (-OH).

3-Bromo-4,5-dimethoxybenzyl Chloride (IVh) : yield 94.5%, m.p. 58-59°.

3. Synthesis of substituted benzylacetamidomalonates (V).

Diethyl(3-Bromo-4-hydroxy-5-methoxy)benzylacetamidomalonate (Ve).[1]

This compound is synthesized according to the literature.

Using the same procedure of synthesis and starting with the adequately substituted benzylchlorides (IVd, IVf, IVg, IVh, IVj), the following diethyl acetamidomalonate are obtained (yield and m.p.) :

Diethyl (2-Bromo-4-hydroxy-5-methoxy)benzylacetamidomalonate (Vd) : 91.5%, 118-119°, purity by potentiometric titration, 98.3% (-OH).

Diethyl (2,3-Dibromo-4-hydroxy-5-methoxy)benzylacetamidomalonate (Vf) : 55.5%, 159-161°, purity, 100% (-OH).

Diethyl (2-Bromo-4,5-dimethoxy)benzylacetamidomalonate (Vg) : 83.5%, 167-168°.

Diethyl (3-Bromo-4,5-dimethoxy)benzylacetamidomalonate (Vh) : 92%, 118-119.5°.

Diethyl (3-Bromo-4,5-dihydroxy)benzylacetamidomalonate (Vj) : 44%, 203-204°, purity, 100% (OH).

4. Syntheses of substituted phenylalanines (I).

The malonic derivatives (Vd, Vf, Vg, Vh & Vj) are refluxed with hydrochloric acid in acetic acid[1] at 90-100° (Table II).

Table II.

Malonic derivatives	conditions of hydrolysis
Vd	4 hr at 100° with HCl 6N
Vf	9 hr at 90° with HCl 6N
Vg	20 hr at 90° with HCl 4N
Vh	20 hr at 90° with HCl 4N
Vj	6 hr at 100° with HCl 12N

After concentration to dryness, the residue is dissolved in water, discoloured, purified via the free base and then treated with hydrochloric acid to yield :

β -(2-Bromo-4-hydroxy-5-methoxyphenyl)alanine.HCl (Id) : yield, 57%.

β -(2,3-Dibromo-4-hydroxy-5-methoxyphenyl)alanine.HCl (If) : yield, 41%.

β -(2-Bromo-4,5-dimethoxyphenyl)alanine.HCl (Ig) : yield, 51%.

β -(3-Bromo-4,5-dimethoxyphenyl)alanine.HCl (Ih) : yield, 48%.

β -(3-Bromo-4,5-dihydroxyphenyl)alanine.HCl (Ij) : yield, 60%.

c) Scheme C.

β -(2-Bromo-5-hydroxy-4-methoxyphenyl)alanine .HBr (Ib). - β-(3-Hydroxy-4-methoxyphenyl)alanine (10.55 g.)[19] *dissolved in 99% acetic acid (100 ml.) is treated dropwise with a 1 molar solution of bromine in acetic acid (50 ml.). The reaction mixture is then concentrated under vacuo. The residue is dissolved in dioxane and precipitated in anhydrous ether to yield (Ib) (13.7 g., 74%). pK_a 9.05.*

The position 2 of the bromine atom is evident from the NMR values of the aromatic protons and from the demethylation of the molecule. This amino-acid (Ib) is demethylated in 40% hydrobromic acid at 145° yielding β-(2-bromo-4,5-dihydroxyphenyl)alanine.HBr (Ii).

As previously described for β-(3-bromo-4-hydroxy-5-methoxyphenyl)-DL-alanine[1] *and for 2,3- and 2,5-dihydroxyphenyl-DL-alanine,*[20] *this amino-acid (Ii) is resolved, on whatman n° 1, into its enantiomers (Rf 0.41 and 0.59) using n-butanol saturated with 3 N-hydrochloric acid as solvent. The same Rf values are obtained with an authentical sample of β-(2-bromo-4,5-dihydroxyphenyl)alanine, synthesized by demethylation of β-(2-bromo-4-hydroxy-5-methoxyphenyl)alanine (Id) or by bromination of Dopa.*[16]

β-(2,3,6-Tribromo-4,5-dihydroxyphenyl)alanine (In). - *Dopa (11.82 g.) in 99% acetic acid (300 ml.) is treated dropwise with a 1 molar solution of bromine in acetic acid (185 ml.). After 20 hr. stirring at 25° and 4 hr. at 40°, the reaction mixture is discoloured on charcoal and the pH of the solution is brought to 5.75 with a saturated aqueous sodium bicarbonate solution. The crystallyzed amino-acid is filtered, washed with cold water and dried to yield (In) (20.9 g., 80%).* pK_a *5.8.*

β-(2,6-Dibromo-3,4-dihydroxyphenyl)alanine (Im). - *A molar solution of bromine in acetic acid (120 ml.) is added to Dopa (11.8 g.) dissolved in 99-100% acetic acid (300 ml.).*

This mixture is stirred 24 hr. at room temperature and 5 hr. at 40°. After purification on charcoal the pH is brought to 5.75 with a concentrated solution of sodium bicarbonate. The dibromo amino-acid crystallizes at 4°C and is dried at 45° over phosphorous pentoxide under vacuo (Im) (5.3 g., 25%). pK_a *7.2.*

This amino-acid was previously described[16] *as X, 6-dibromodopa. Position 2 is here assigned to the bromine atom designed as X. This was done by chromatographic comparison on Whatman n° 1 (solvent : n-butanol saturated with 3 N-hydrochloric acid) with β-(2,3-dibromo-4,5-dihydroxyphenyl)alanine (Ik) obtained by demethylation of the 5-methoxy analog (If) : Rf 2,6-dibromo-3,4-dihydroxy- (Im) 0.76 and 0.78 (separation of the enantiomers). Rf 2,3-dibromo-4,5-dihydroxy- (Ik) 0.62.*

Table III.

pK_a	✱	n°	R_F	m.p.	Elemental analyses						U.V. spectra (water)	
					calc. %			Found %				
					C	H	N	C	H	N	λ_{max} (nm)	ε
8.4	A	Ia	0.90	151-153°	36.8	4.0	4.3	36.4	3.9	4.6	286, 276 & 251	1,839; 1,711 & 315
9.05	C	Ib	0.70	213-214°	36.8	4.0	4.3	36.9	4.1	4.3	285 & 260	2,788 & 621
—	B	Id	0.75	168-170°	36.8	4.0	4.3	36.4	3.9	4.4	285 & 259	2,688 & 656
7.25	B	If	0.78	225-227°	29.6	3.0	3.5	29.6	3.1	3.6	291, 288 & 264	2,222; 2,104 & 815
—	B	Ig	0.83	232-233°	38.8	4.4	4.1	38.6	4.6	4.2	283 & 260	2,880 & 774
—	B	Ih	0.92	232-234°	38.8	4.4	4.1	39.1	4.4	3.9	282, 276 & 252	1,663; 1,591 & 320
8.05	Ib	Ii	0.41 & 0.59	228-230°	30.3	3.1	3.9	30.5	3.2	4.0	286 & 258	3,015 & 550
7.6	B	Ij	0.69 & 0.75	129-133°	34.6	3.5	4.5	34.6	3.4	4.4	283, 260 & 253	2,019; 1,989 & 339
7.2	C	Im	0.76 & 0.78	180-181°	30.4	2.6	3.9	30.3	2.7	3.8	292 & 263	2,380 & 1,090
5.8	C	In	0.81✱✱	190-191°	24.9	1.9	3.2	25.0	2.0	3.2	295, 290 & 275	1,895; 1,709 & 1,228

✱ *method of synthesis*

✱✱ *0.52 and 0.57 with n-butanol-acetic acid-water (4:1:5)*

Table IV.

n°	Purity (✱) %	M.M.R. spectra : ppm and (J cps)					
		Solvent	Aromatics	-CH-	$-O-CH_3$	$-O-CH_3$	$-CH_2-$
Ia	95.6 (-COOH) 95.5 (HCl) 95.6 (-OH)	D_2O + DCl	7.10 d (2.0) 6.89 d (2.0)	4.39 dd (7.4 & 6.0)	3.85 s	—	3.20 d (6.0) 3.18 d (7.4)
Ib	97.0 ($-NH_2$)	D_2O	7.17 s 6.87 s	4.35 dd (7.1 & 6.3)	3.82 s	—	3.26 d (6.3) 3.21 d (7.1)
Id	97.3 ($-NH_2$)	D_2O	7.15 s 6.93 s	4.39 dd (7.4 & 6.0)	3.89 s	—	3.33 d (6.0) 3.28 d (7.4)
If	99.5 ($-NH_2$)	D_2O	6.90 s —	4.37 dd (8.7 & 6.7)	3.90 s	—	3.37 d (6.7) 3.30 d (8.7)
Ig	98.5 ($-NH_2$)	D_2O + $DMSO-d_6$	7.20 s 7.01 s	4.31 dd (8.7 & 6.7)	3.81 s	3.81 s	3.31 d (6.7) 3.29 d (8.7)
Ih	99.0 ($-NH_2$)	D_2O	7.16 d (2.0) 7.02 d (2.0)	4.37 dd (7.2 & 6.0)	3.90 s	3.82 s	3.25 d (6.0) 3.24 d (7.2)
Ii	98.5 ($-NH_2$)	D_2O	7.11 s 6.87 s	4.35 dd (7.1 & 6.3)	3.82 s	—	3.26 d (6.3) 3.21 d (7.1)
Ij	99.0 ($-NH_2$)	D_2O	6.98 d (2.1) 6.78 d (2.1)	4.30 dd (7.5 & 5.9)	—	—	3.15 d (5.9) 3.12 d (7.5)
Im	99.0 ($-NH_2$)	D_2O + $DMSO-d_6$	6.97 s —	4.28 t (7.8)	—	—	3.36 d (7.8) 3.36 d (7.8)
In	100.0 ($-NH_2$)	D_2O + DCl	— —	4.39 t (8.0)	—	—	3.63 d (8.0) 3.58 d (8.0)

✱ *by potentiometric titration.*

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