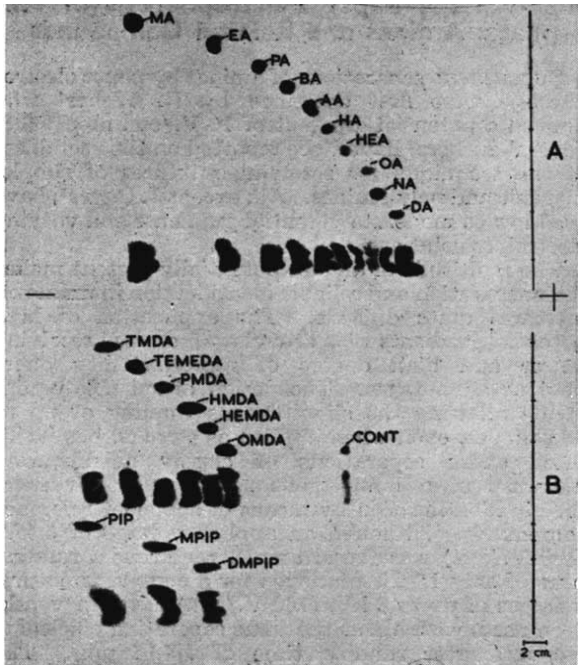

Fig. 2. Electrophoresis of some homologous series of amines and piperazines. Conditions: Same as in Fig. 1, except for cooling water temperature 12°C., and running time 17 minutes. Mono-amines: *AA*, amylamine; *DA*, decylamine; *HA*, hexylamine; *HEA*, heptylamine; *NA*, nonylamine; *OA*, octylamine; Diamines: *CONT*, contaminant; *HEMDA*, heptamethylene-diamine; *HMDA*, hexamethylene-diamine; *OMDA*, octamethylene-diamine; *PMDA*, pentamethylene-diamine; Piperazines: *DMPIP*, trans—2,5, dimethylpiperazine; *MPIP*, 2-methylpiperazine; *PIP*, piperazine

the migration-rates of the compounds in the mixture, compared with the individual migration rates, is a concentration effect due to heavier loading and mutual ionic interference.

It may be added that the high-voltage technique has been recently also successfully applied to mixtures of polyamines, glyoxalines and pyrroles.

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BIOCHEMISTRY

Synthesis of a Protected Heptapeptide Related to the Histidine Analogue of Hypertensin II

Hypertensin II or isoleucine hypertensin II, a pressor peptide with the amino-acid sequence Asp.Arg.Val.Tyr.Is.His.Pro.Phe. has been synthesized along classical lines by two groups of investigators[1].

This octapeptide hormone, in contrast to oxytocin in which the intact 20-membered disulphide ring structure is basic for activity[2], is a straight chain polypeptide. Due to its simple structure and important biological function, hypertensin is very suitable for studying the relationship between chemical structure and biological activity. Moreover, the accumulated evidence for the existence of a peptide-like material with hypotensive activity[3] stimulates new interest for the synthesis of hypertensin analogues.

This communication reports the synthesis of the protected heptapeptide carbobenzoxy-im.benzyl-L-histidyl-L-valyl-L-tyrosyl-L-isoleusyl-im.benzyl-L-histidyl-L-prolyl-L-phenylalanine benzyl ester, related to the histidine analogue of hypertensin II.

The synthesis of the histidine hypertensin analogue, in which the arginine residue is replaced by histidine, has been of especial interest since the finding that the basicity of the amino-acid residue in the side chain of vasopressin[4] has a strong influence on the degree of pressor activity. Consequently, it was considered of interest to investigate whether replacement of the strongly basic arginine residue of hypertensin II by a weakly basic amino-acid (histidine) influenced the pressor activity of the hormone.

Asp.Arg.Val.Tyr.Is.His.Pro.Phe.
Hypertensin II
Asp.His.Val.Tyr.Is.His.Pro.Phe.
Histidine hypertensin II

Recently developed methods were used for the preparation of the protected heptapeptide. Thus, trityl-L-valine[7] was coupled with L-tyrosine methyl ester, by the dicyclohexyl-carbodiimide method and the oily product was detritylated[5] to give L-valyl-L-tyrosine methyl ester hydrocloride (I), in 40 per cent yield, $[\alpha]_D^{25}+32°$ (*c.*, 1 in methanol), R_F 0·91 in *n*-butanol-acetic acid-pyridine-water system[8]. Calculated for $C_{15}H_{23}N_2O_4Cl$: N 10·82, Cl 13·79; found: N 10·50, Cl 13·51.

Carbobenzoxy-im.benzyl-L-histidine[6] was coupled with I in dimethylformamide using dicyclohexyl-carbodiimide as the condensing agent[9]. The product, carbobenzoxy-im.benzyl-L-histidyl-L-valyl-L-tyrosine methyl ester (II) was precipitated with water, yield 73 per cent, m.p. 192–194°C. (all melting points are uncorrected). Calculated for $C_{36}H_{41}N_5O_7$: C 65·93, H 6·30, N 10·68; found: C 65·82, H 6·25, N 10·63.

Hydrolysis of II with *N*-sodium hydroxide followed by acidification with acetic acid produced the acid, carbobenzoxy-im.benzyl-L-histidyl-L-valyl-L-tyrosine (III), in 80 per cent yield, m.p. 185–186°C. Calculated for $C_{35}H_{39}N_5O_4$: C 65·50, H 6·12, N 10·90; found: C 65·45, H 6·10, N 10·99.

Carbobenzoxy-L-isoleucine[10] was coupled by the mixed anhydride method with im.benzyl-L-histidine benzyl ester *p*-toluenesulphonate[9] and the product, carbobenzoxy-L-isoleucyl-im.benzyl-L-histidine benzyl ester[11] was saponified with *N*-sodium hydroxide Acidification with acetic acid afforded the acid, carbobenzoxy-L-isoleucyl-im.benzyl-L-histidine (IV), $[\alpha]_D^{26}+23·5°$ (*c.*, 1·1 in glacial acetic acid), m.p. 172–174°C. (softens at 138–140°C.). Calculated for $C_{27}H_{32}N_4O_5$: C 65·83, H 6·54, N 11·33; found: C 65·40, H 6·32, N 11·02.

Carbobenzoxy-L-proline was coupled by the mixed anhydride method with L-phenylalanine methyl ester and the oily product was hydrogenated in the presence of palladium oxide, or palladium chloride in methanol containing one equivalent of hydrogen chloride. The product, L-prolyl-L-phenylalanine methyl ester hydrochloride (V) was obtained in 73 per cent yield, m.p. 158–159°C. (reported[1] 157–158°C., 162–164°C.).

Compound IV was suspended in methylene chloride and dissolved by addition of one equivalent of triethylamine. To this solution the equivalent amount of V dissolved in methylene chloride was

added, followed by addition of carbodiimide. After 12 hr. the mixture was filtered and the solvent evaporated. The residue was taken up in ethyl acetate and treated as previously described[11]. The syrupy tetrapeptide ester was saponified to give the acid (amorphous), which was further hydrogenated in the presence of palladium oxide in ethanol. When the evolution of carbon dioxide ceased, the hydrogenation was discontinued, and water was added; on heating all the precipitate dissolved. The catalyst was filtered and the solution evaporated *in vacuo*. The residue was dissolved in acetone and upon addition of ether the product L-isoleucyl-im.benzyl-L-histidyl-L-prolyl-L-phenylalanine (VI) precipitated, $[\alpha]_D^{26} + 19°$ (*c.*, 1 in glacial acetic acid), R_F 0·76 in *sec.* butanol-formic acid-water[12] 0·76; R_F 0·78 in *n*-butanol-acetic acid-pyridine-water. Calculated for $C_{33}H_{42}N_6O_5 \cdot 2H_2O$: C 62·0, H 7·26, N 13·15; found: C 62·0, H 7·27, N 13·20.

Compound VI upon treatment with benzyl alcohol in the presence of *p*-toluenesulphonic acid was converted to its benzyl ester di-*p*-toluene-sulphonate almost in quantitative yield, R_F 0·95 in *n*-butanol-acetic acid-pyridine-water. Calculated for $C_{54}H_{64}N_6O_{11}S_2 \cdot H_2O$: N 8·1; found: N 7·85. This esterification process has been also successfully applied in the synthesis of lysyl peptide benzyl esters during our recent studies[13] dealing with the inhibitory role of phosphoserine adjacent to a lysine residue.

The above tetrapeptide benzyl ester (1·48 gm.) was condensed with tripeptide III (0·8 gm.) by the carbodiimide method in dimethylformamide. The solvent was then evaporated, the residue taken up in ethyl acetate, washed, and the ethyl acetate distilled off. The remaining product, carbobenzoxy-im.benzyl-L-histidyl-L-valyl-L-tyrosyl-L-isoleucyl-im.benzyl-L-histidyl-L-prolyl-L-phenylalanine benzyl ester was filtered by addition of ether. Yield 0·98 gm. (60 per cent crude product). Recrystallized twice from ethanol-ether melted at 145–147°C., $[\alpha]_D^{25} -27°$ (*c.*, 1 in glacial acetic acid). Calculated for $C_{75}H_{85}N_{11}O_{11}$: C 68·47, H 6·50, N 11·71; found: C 68·20, H 6·35, N 11·56. Paper chromatography of the hydrolysate revealed the expected amino-acids in about equal amounts, with the exception of im.benzyl-L-histidine, which gave a more intense spot.

Further details, and the final attempt towards the total synthesis of the histidine analogue of hypertensin II, will appear in a subsequent communication elsewhere.

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Chromatographic Identification of the Antilipæmic *X* Factor of the Unsaponifiable Fraction of Crude Vegetable Oils

SEVERAL workers[1,2] hold that there is some unknown factor in crude corn oil and other vegetable oils which are more effective in lowering the elevated serum cholesterol and phospholipid levels in atherosclerosis rather than purified essential fatty acids alone. Jensen *et al.*[3] reported that crude corn oil contains an unidentified nutritional factor which is more beneficial in increasing the egg weight and hatchability in the hen than essential fatty acids. Recently, Grande *et al.*[4] have shown that there is a factor *X* present in the unsaponifiable fraction of crude corn oil, which can be taken as a better antilipæmic agent than purified essential fatty acids.

It has recently been reported from this laboratory that glucose *cyclo*acetoacetate, a product of condensation of glucose with ethyl acetoacetate, which has been shown by Gonzales and Aparacio[5] to be 2-tetrahydroxy butyl, 5-methyl, 4-carbethoxy furan, on hydrolysis with 2*N* hydrochloric acid gives rise to 1 : 2 dienol glucose[6]. Hydrolysed glucose *cyclo*acetoacetate has also been shown to have a pronounced effect in maintaining the normal levels of cholesterol, and phospholid, and also the C/P ratio in serum and tissues in different kinds of experimental atherosclerosis (ref. 7–9). It is more effective than the essential fatty acids, vitamin B_{12} or inositol. It has also recently been observed that this substance gives a blue colour with molybdenum reagent in acid medium[6] and this test is positive with the unsponifiable fraction of crude linseed oil[9]. Further, in combination with essential fatty acids, this compound has been found to be similar to crude linseed oil in that it lowers the C/P ratio in atherosclerosis induced by saturated fats in rabbits better than the essential fatty acids alone.

In view of the fact that 1 : 2 dienol glucose shows a greater beneficial effect than that caused by essential fatty acids or vitamins B_{12} and because that effect is similar, in some way or other, to that of crude linseed oil, groundnut oil or sesame oil in different kinds of hypercholesterolæmia and experimental atherosclerosis induced by saturated fats[7,9], it has been thought desirable to study whether the unknown *X* factor of the crude vegetable oil bears any resemblance with the 1 : 2 dienol glucose when subjected to circular paper chromatography.

The unsaponifiable fractions of corn oil, groundnut oil, linseed oil and dalda (hydrogenated groundnut oil) were extracted by the method of Grande *et al.*[4] The fractions were purified by removing the colouring matters and other impurities with 'Norite' followed by fuller's earth. 20 μl. of the purified unsaponifiable fraction was spotted on Whatmann No. 1 filter paper of 11 in. diameter with an inner circle of 1 in. radius. The technique of Nath and Bhattathiry[6] using butanol/acetic acid/water (4 : 1 : 5) was followed and the colour developed with phosphomolybdic acid reagent.

Fig. 1 shows that 1 : 2 dienol glucose has the same R_F value (0·92) as that of the *X* factor of the unsaponifiable fractions of corn oil and groundnut oil. Dalda (hydrogenated groundnut oil) did not, however, show the presence of this band, thus indicating that the potent *X* is destroyed by hydrogenation. It is interesting to note that glucose *cyclo*acetoacetate on acid hydrolysis for more than 15 min. gives rise to another band lower than that of 1 : 2 dienol glucose;