the treatment with carbon, the color reaction of the culture filtrate with NaOH and $FeCl_3$ became negative. The carbon which adsorbed the pigment was then extracted with MeOH, and MeOH extract was evaporated *in vacuo*. The residue thus obtained was suspended in water to remove soluble carbohydrate portion, and the water-insoluble fraction was collected by centrifugation as a red powder (180 mg). The powder was then treated with acetone. The acetone-insoluble orange powder obtained in a very small amount did not melt below 330°, and was not further examined. The acetone-soluble fraction was examined by TLC using silica gel as the adsorbent and chloroform as the solvent. Two yellow spots were detected on the plate. One spot, whose *Rf* was the same as that of macrosporin, was extracted with acetone and identified with macrosporin by mixed fusion. Another yellow spot was also extracted with acetone and recrystallized from EtOH to give yellow needles of mp 182°. IR ν_{max}^{KBr} cm^{-1}: 1588, 1601 (aromatic ring), 1629 (chelated carbonyl), 1672 (non-chelated carbonyl). Mass Spectrum *m/e*: 268 (M^+). This compound was identified with the authentic sample of 6-methylxanthopurpurin 3-methyl ether (II) by mixed fusion and comparison of infrared spectra.

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Comparative Studies on Angiotensins. III.[1] Structure of Fowl Angiotensin and Its Identification by DNS-Method[2]

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The structure of native angiotensin I, produced by incubating homologous renin with homologous substrate, is Asp[1]-Arg[2]-Val[3]-Tyr[4]-Ile[5]-His[6]-Pro[7]-Phe[8]-His[9]-Leu[10] in the human[4] and rat.[1b] Val[5]-angiotensin I was obtained when ox plasma was incubated with rabbit renin.[5]

Fowl angiotensin was different from Asp[1]-Ile[5]-angiotensin I, II, or Asn[1]-Val[5]-angiotensin II by its SE-Sephadex chromatographic behavior, ratio of oxytocic to pressor activity (O: P ratio), and susceptibility to carboxypeptidase (CPase) A.[1a] We have identified fowl angiotensin as Asp[1]-Arg[2]-Val[3]-Tyr[4]-Val[5]-His[6]-Pro[7]-Phe[8]-Ser[9]-Leu[10] by its amino acid composition and characteristics of its DNS- (1-dimethyl amino naphthalene 5-sulphonyl-) derivatives.

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Experimental

Fowl (white leghorn) angiotensin was prepared by incubating the kidney extract with 2670 ml of plasma from 40 birds following the same method used previously.[1a] The crude active principle of 330 μg (equivalent to Asn[1]-Val[5]-angiotensin II) was obtained, and purified by gel filtration, ion exchange, and droplet counter current (DCC) chromatography (Chart 1).

fowl plasma (2670 ml), fowl kidney extract

(1) incubation of plasma with kidney extract
(2) Dowex 50W-X2 (NH_4^+) chromatography
(3) Sephadex G-25 chromatograpy
(4) SE-Sephadex (NH_4^+) chromatography
(5) droplet counter current chromatography
(6) SE-Sephedex (NH_4^+) chromatography

active principle (major peak in step 5) (126 μg equivalent to Asn[1]-Val[5]-angiotensin II)

Chart 1. Formation and Purification of Fowl Angiotensin

Details of purification, dansylation, and determination of the pressor and oxytocic activity were reported previously.[1a,b]

Result

The active principle was separated into two peaks by DCC chromatography. They were different in O: P ratio or susceptibility to CPase A (Table I).

Table I. Two Peaks on Droplet Counter Current Chromatogram

	Peak 1	Peak 2
Distribution (% of total activity)	30—40	60—70
O:P ratio (%)[a]	24.8	<6.1
Protease susceptibility[b] (% decrease in activity)		
Trypsin	80.0	66.9
CPase A	5.0	80.4

a) Ratio of oxytocic to pressor activity. That of Asn[1]-Val[5]-angiotensin II is 100%.[1a]
b) Detail of the enzyme treatment was reported previously.[1a]

The major peak (peak 2) was examined further. Active principle of 126 μg equivalent to Asn[1]-Val[5]-angiotensin II was obtained as a single spot on thin layer chromatogram after dansylation. *Rf* value of this DNS-fowl material was different from N-DNS-Asp[1]-O-DNS-Tyr[4]-Ile[5]-angiotensin I or II. Amino acid composition obtained by an amino acid analyzer (JEOL 5AH) was His 1, Arg 1, Asp 1, Ser 1, Pro 1, Val 2, Leu 1, Tyr 1, and Phe 1.

Treatment of this fowl material with CPase A formed an equivalent amount of leucine and traces of serine and phenylalanine. The lack of histidine after reduction with sodium in liquid ammonia (Birch reduction) was supported to have a His-Pro bond. Hydrolysate of the DNS-material with 6N-HCl contained DNS-Asp, and O-DNS-Tyr. The DNS-material was cleft into two fragments by trypsin. One was identical with DNS-Asp-Arg, and N-terminal of the other fragment was valine. Treatment with chymotrypsin of the DNS-material gave also two fragments, one of which was Ser-Leu. These results indicate amino acid sequence from the N-terminal was Asp-Arg-Val-, and to C-terminal was -Phe-Ser-Leu.

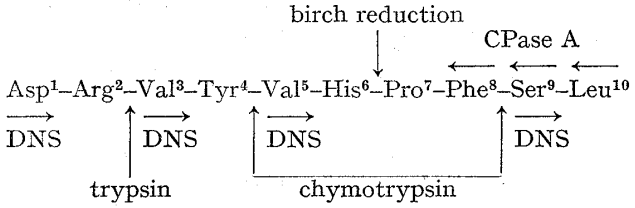

Chart 2. The Amino Acid Sequence of Fowl Angiotensin Decapeptide, and the Procedures to Identify it by Its DNS-Derivatives

Chymotryptic treatment without dansylation produced there fragments. They were identified after dansylation as DNS-Asp-Arg-Val-O-DNS-Tyr, DNS-Val-His-Pro-Phe, and DNS- Ser-Leu. These procedures with the entire amino acid sequence are shown in Chart 2.

Discussion

Fowl angiotensin decapeptide has 5-valine and 9-serine. Its amino acid sequence is different from mammalian angiotensins I, but its oxytocic activity is as weak as theirs. Fowl angiotensin showed pressor activity probably after being converted into Asp^{1}-Val^{5}-angiotensin II octapeptide, when injected intravenously into the rat. The converting enzyme system of fowl angiotensin is unknown, but dialysis of the kidney extract and plasma against 4.9 mM ethylenediamine tetraacetic acid inhibits this enzyme in mammals.[1a,b] Further study is necessary to determine the active form of fowl angiotensin.

Fowl angiotensin decapeptide was split easily 10-leucine but not 9-serine by CPase A, and lost 80.4% of pressor activity. Formed nonapeptide may have about one fifth of pressor activity, probably due to poor conversion into octapeptide. Peak 1 in DCC chromatogram (Table I) was probably a product by some proteases in the kidney extract, because the active principle produced by incubating plasma (plasma renin with angiotensinogen) without the kidney extract showed a single peak at the position of peak 2 in DCC chromatogram. Analysis of peak 1 is under way.

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