It is concluded that the 4 sub-units cannot all be the same, since the minimum molecular weight is identical with the molecular weight.

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[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND ZOÖLOGY, COLUMBIA UNIVERSITY]

Leucine Content of Proteins and Foodstuffs[1,2]

BY ERWIN BRAND, FRANCIS J. RYAN AND EUGENE M. DISKANT

In a recent publication[3] we have described a simple microbiological method for the accurate determination of leucine with the aid of the "leucineless" strain of *Neurospora crassa* developed by Regnery[4] in Beadle's laboratory. The reliability of the microbiological method was clearly established by a comparison with the methods involving solubility product and isotope dilution. The proportions of leucine found in crystalline egg albumin, gelatin and crystalline horse hemoglobin were practically the same by these fundamentally different methods, identical preparations having been analyzed in each case. For β-lactoglobulin we reported[3] a leucine content of 15.4%. Since then another sample of β-lactoglobulin (obtained from Dr. G. Haugaard) has yielded in the hands of Dr. G. L. Foster[5] 15.7% by the isotope dilution method. This increases our confidence in the results by the *Neurospora* method. We have previously[3] pointed out that the bioassay for leucine with *Lactobacillus arabinosus*, as carried out by Kuiken, *et al.*,[6] gave results for gelatin and casein only slightly lower than those reported by us.[3] Schweigert, *et al.*,[7] have since obtained a leucine value of 9.6% for casein by the *L. arabinosus* method in good agreement with our value of 9.8%.[3]

Reliable values for the leucine content of proteins obtained with chemical methods are available only for silk fibroin, gelatin and egg albumin which were obtained by the solubility product method[8] and for horse hemoglobin and β-lactoglobulin by the isotope dilution method.[5] Most of the older values for leucine in the literature are of questionable reliability. Moreover, they usually refer to the sum of the leucine and isoleucine, although this is not always explicitly stated nor recognized. Since the *Neurospora* method yields results which confirm the chemical methods in the case of the above proteins, it was thought desirable to extend our knowledge of protein composition by determining the leucine content of a number of common proteins and especially of certain crystalline preparations.

The data are presented in Table I. The leucine values previously reported[3,9] are included. Except as especially noted, the values are on a moisture-, ash- and sulfate-free basis.

Protein Preparations

We are indebted to many workers in the field of protein chemistry for samples of their preparations, as indicated below for the individual proteins.

Blood Proteins.—Most of the proteins were prepared[10] by the Department of Physical Chemistry, Harvard Medical School. Human serum albumin (no. 42) and bovine serum albumin (no. 456) were crystalline preparations (*cf.* ref. 9) and were practically homogeneous by electrophoretic and ultracentrifuge criteria (*cf.* ref. 11). Horse serum albumin A (the carbohydrate containing fraction, so designated by Kekwick)[12] was prepared by Dr. Hans Neurath, while horse serum albumin B (the carbohydrate free fraction, (*cf.* ref. 12)) was prepared by Dr. Manfred Mayer of the Department of Medicine, College of Physicians and Surgeons, according to the method of Adair and Robinson.[13]

Human serum γ-globulin (no. 36, II-1, *cf.* refs. 9, 11) was electrophoretically uniform, but was not homogeneous with respect to size (*cf.* ref. 9, Table II, footnote 2 and ref. 14). The human β-globulin (no. III-2, Prep. GL291, refs. 9, 11) was a complex mixture of human serum globulins about two-thirds of which had the mobility associated with β-globulin.

Human fibrinogen (no. 81, RI, *cf.* refs. 9) was clottable to the extent of about 87% of its protein content; the value reported is calculated on the assumption that the content of total nitrogen is the same in human fibrinogen as in human fibrin

(1) Part of the work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University.

(2) Some of the data in this paper were presented before the Division of Biological Chemistry at the 106th meeting of the American Chemical Society, Pittsburgh, Pa., Sept., 1943.

(3) F. J. Ryan and E. Brand, *J. Biol. Chem.*, **154**, 161 (1944).

(4) D. C. Regnery, *ibid.*, **154**, 151 (1944).

(5) G. L. Foster, *ibid.*, in press.

(6) K. A. Kuiken, W. H. Norman, C. M. Lyman, F. Hale and L. Blotter, *ibid.*, **151**, 615 (1943).

(7) B. S. Schweigert, J. M. McIntire, C. A. Elvehjem and F. M. Strong, *ibid.*, **155**, 183 (1944).

(8) S. Moore and W. H. Stein, *ibid.*, **150**, 113 (1943).

(9) E. Brand, B. Kassell and L. J. Saidel, *J. Clin. Invest.*, **23**, 437 (1944).

(10) These proteins were prepared from blood collected by the American Red Cross, under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

(11) E. J. Cohn, J. L. Oncley, L. E. Strong, W. L. Hughes, Jr. and S. H. Armstrong, Jr., *J. Clin. Invest.*, **23**, 417 (1944).

(12) R. A. Kekwick, *Biochem. J.*, **32**, 552 (1938).

(13) G. S. Adair and M. E. Robinson, *ibid.*, **24**, 993 (1930).

(14) J. W. Williams, M. L. Peterman, G. C. Colovos, M. B. Goodloe, J. L. Oncley and S. H. Armstrong, Jr., *J. Clin. Invest.*, **23**, 433 (1944).

(16.9%). The human fibrin was prepared by Dr. H. B. Vickery from a lot of fibrinogen which had clotted spontaneously. The bovine fibrin (*cf.* ref. 9 Table IV) was a commercial preparation, the value given is based upon a total nitrogen content of 17.0%.

The horse hemoglobin (twice recrystallized) was prepared by Dr. G. L. Foster[5] and the sample of human hemoglobin by Dr. R. K. Cannan.

Enzymes and Hormones.—The crystalline insulin was prepared by Dr. V. du Vigneaud (*cf.* ref. 3). Ribonuclease, chymotrypsinogen, α,β,γ-chymotrypsin, trypsinogen and trypsin were crystallized by Dr. M. Kunitz. Pepsin D was a crystalline sample prepared by Dr. R. M. Herriott and subsequently denatured. For other analytical data on these preparations see refs. 15 and 16.

Animal Proteins.—Gelatin O was prepared by the late Dr. T. B. Osborne and obtained from Dr. H. B. Vickery. Gelatin Bg and egg albumin Bg were obtained from the late Dr. M. Bergmann and Drs. W. H. Stein and S. Moore (*cf.* refs. 3 and 17). Egg albumin C, crystallized once and denatured, was prepared by Dr. H. O. Calvery. Elastin was prepared by Drs. W. H. Stein and E. G. Miller (*cf.* ref. 18). Vitellin was prepared by Dr. B. H. Nicolet and Mr. L. J. Saidel. Bence Jones protein was prepared by one of the authors (E. B.).

Vegetable Proteins.—With the exception of the peanut proteins these proteins were prepared at the Connecticut Agricultural Experiment Station, New Haven, and were obtained from Dr. H. B. Vickery. The sample of edestin was prepared in 1928. The sample of glycinin (precipitated by dialysis) was prepared by the late Dr. T. B. Osborne in 1920. The Zein was prepared in 1923; the Gliadin in 1939. The cotton seed globulin no. 16 was prepared in 1916, and cotton seed globulin no. 27 in 1927 by Mr. L. S. Nolan. The Arachin + Conarachin was prepared by Johns and Jones from peanuts with hulls, the total peanut globulin (Johns, prep. No. 201–1917) from Virginia peanuts. These peanut protein preparations were gifts to the late Dr. T. B. Osborne.

Foodstuffs.—Skim milk powder, dried brewers' yeast and wheat flour were commercial materials.

Ribonuclease[19] is the only protein so far investigated that does not contain *l*(+)-leucine. We have controlled this observation by adding small amounts of *l*-leucine to the hydrolyzates, whereupon prompt mold growth resulted. The negative results of the leucine assay in this case are therefore not due to the presence of special inhibitors in hydrolyzates of ribonuclease. Regnery[4] has shown that the "leucineless" mutant of *Neurospora* cannot utilize the unnatural form of leucine for growth unless small amounts of natural leucine are also present (*cf.* ref. 3). The above experiments, therefore, indicate only the absence of *l*-leucine in ribonuclease; the absence of *d*-leucine remains to be established.

TABLE I

LEUCINE CONTENT OF PROTEINS[a]

Proteins	Protein, g. per 100 g.	Protein, moles per 10^5 g.	Residues per mole, protein: Residues	Residues per mole, protein: Assumed molecular weight
Blood proteins				
Human serum albumin, no. 42	11.9	90.7	64	70,000
Bovine serum albumin, no. 456	13.7	104.4	73	70,000
Horse serum albumin A	13.0	99.1	69	70,000
Horse serum albumin B	10.1	77.0	54	70,000
Human β-globulin, no. GL291	8.9	67.9		
Human γ-globulin, no. 36	9.3	70.9	122	171,000
Human fibrinogen, no. 81 RI	7.1	54.2		
Human fibrin	7.1	54.2		
Bovine fibrin	7.5	57.2		
Human hemoglobin	14.7	112.1	75	66,700
Horse hemoglobin	15.7	119.7	80	66,700
Enzymes and hormones				
Ribonuclease	0	0	0	
Insulin	13.4	101.4	45	44,600[b]
Chymotrypsinogen, no. II	10.4	79.3	29	36,700
α-Chymotrypsin, no. II	9.1	69.4		
β-Chymotrypsin	9.1	69.4		
γ-Chymotrypsin, no. II	8.5	64.8		
Trypsinogen	7.6	57.9		
Trypsin	7.8	59.5		
Pepsin, no. D	10.4	79.3	27	34,400[c]
Animal proteins				
Silk fibroin[d]	0.8	6.1		
Gelatin O	3.6	27.4		
Gelatin Bg	3.6	27.4		
Elastin	8.6	65.6		
Egg albumin Bg	9.6	73.2	32	43,000
Egg albumin C	9.9	75.5	32	43,000
β-Lactoglobulin (*cf.* ref. 21)	15.6	118.9	50	42,020
Casein (vit. free labco)	9.8	74.7		
Bence-Jones protein[e]	7.6	57.9		
Vitellin[e]	11.3	86.2		
Vegetable proteins				
Edestin	7.4	56.4		
Gliadin[e]	6.5	49.6		
Zein[e]	15.4	117.4		
Glycinin[e]	8.4	64.0		
Arachin + conarachin[e]	8.0	61.0		
Total peanut globulin[e]	8.1	61.8		
Cotton seed globulin no. 16[e]	6.8	51.8		
Cotton seed globulin no. 27[e]	6.4	48.8		
Foodstuffs[f]				
Skim milk powder	3.5			
Dried brewers yeast	2.9			
Wheat flour	0.8			

[a] All values are on a moisture-, ash- and sulfate-free basis, except as otherwise indicated. [b] From unpublished analytical data of E. Brand and L. J. Saidel (molecular weight by ultracentrifuge 46,000 (*cf.* ref. 20). [c] From unpublished analytical data of E. Brand and B. Kassell. [d] Taken from Moore and Stein[8] and determined by the solubility product method. [e] Not corrected for ash. [f] Not corrected for moisture and ash.

(15) E. Brand and B. Kassell, *J. Gen. Physiol.*, **25**, 167 (1941).
(16) E. Brand and B. Kassell, *J. Biol. Chem.*, **145**, 359 (1942).
(17) S. Moore and W. H. Stein, *ibid.*, **150**, 113 (1943).
(18) W. H. Stein and E. G. Miller, *ibid.*, **125**, 599 (1938).
(19) M. Kunitz, *J. Gen. Physiol.*, **24**, 15 (1940).
(20) G. L. Miller and K. J. I. Andersson, *J. Biol. Chem.*, **144**, 459 (1942).
(21) E. Brand, L. J. Saidel, W. H. Goldwater, B. Kassell and F. J. Ryan, THIS JOURNAL, **67**, 1524 (1945).

Of all the proteins so far investigated, silk fibroin has the lowest leucine content (0.8%, determined by the solubility product method[8]), gelatin being next with 3.6%.

Summary

The *leucineless* strain of *Neurospora crassa* was used for the determination of leucine in hydrolysates of a number of proteins and of a few foodstuffs by the technique described previously.

Additional evidence for the reliability of the microbiological method has become available in the case of β-lactoglobulin, for which a leucine content of 15.7% has been found by Dr. G. L. Foster by the isotope dilution method as compared to 15.4% by the Neurospora method.

Ribonuclease is the only protein in which no leucine could be detected.

Of all the proteins so far investigated, silk fibroin has the lowest leucine content (0.8%), gelatin being next with only 3.6%.

Leucine accounts for an appreciable part of the molecule in zein (15.4%), bovine (13.7%), human (11.9%) and horse (13.0%) serum albumin, horse (15.7%) and human (14.7%) hemoglobin, insulin (13.4%) and β-lactoglobulin (15.6%).

Chymotrypsinogen, insulin and β-lactoglobulin contain 29, 45 and 50 residues of leucine, respectively, per mole of protein.

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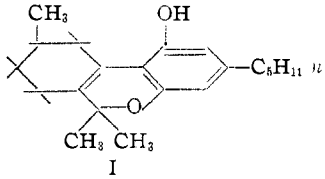
[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, AND THE PHARMACOLOGY DEPARTMENT, CORNELL UNIVERSITY MEDICAL COLLEGE]

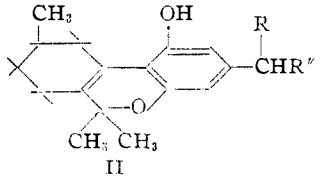
Tetrahydrocannabinol Homologs with a *s*-Alkyl Group in the 3-Position. XVI[1]

BY ROGER ADAMS, K. H. CHEN AND S. LOEWE

Modification of the synthetic tetrahydrocannabinol molecule (I) by changing the substituents in the 6- and 9-positions invariably produced compounds of lower marihuana potency. In the 3-position, however, an increase or decrease in the physiological activity could be attained with variation of the size of the alkyl group. Activity increased progressively from the methyl to the *n*-hexyl and then decreased in the higher homologs.[1,2,3]



I



II

With two exceptions the molecules previously studied had normal groups in the 3-position. Todd[3] described the isoamyl and isohexyl homologs and observed that they had only negligible activity by the Gayer test.

The investigation has now been extended to a series of molecules with alkyl groups containing a secondary carbon attached to the benzene ring (II). In these substances the physiological activity showed a marked increment in value over the corresponding normal alkyl derivatives. In Table I a comparison is given; all products were tested by the procedure previously described.

TABLE I

PHARMACOLOGICAL ACTIVITY OF TETRAHYDROCANNABINOL HOMOLOGS

	3-Substituent	No. of expts.	Potency
1	—C_4H_9-*n*	4	0.37 ± 0.12
2	—C_5H_{11}-*n*	20	1.00 standard
3	—$CH(CH_3)C_3H_7$-*n*	11	1.84 ± 0.13
4	—$CH(C_2H_5)C_3H_7$-*n*	11	1.67 ± 0.33
5	—$CH(CH_3)C_4H_9$-*n*	7	3.65 ± 0.33
6	—$CH(n\text{-}C_3H_7)C_4H_9$-*n*	8	3.17 ± 0.25
7	—C_6H_{13}-*n*	7	1.82 ± 0.18
8	—$CH(CH_3)C_5H_{11}$-*n*	13	4.85 ± 0.82
9	—C_7H_{15}-*n*	10	1.05 ± 0.15
10	—$CH(CH_3)C_6H_{13}$-*n*	10	16.4 ± 3.67
11	—C_8H_{17}-*n*	7	0.66 ± 0.12
12	Tetrahydrocannabinol[4] from cannabidiol (from American hemp)	20	7.3 ± 0.89
13	Natural tetrahydrocannabinol[5] acetate (from charas)	5	14.6 ± 1.05
14	Natural tetrahydrocannabinol[5] by hydrolysis of 13	15	7.8 ± 0.47
15	Pulegone + 5-(1-methylbutyl)-resorcinol	5	0.45 ± 0.05
16	Pulegone + 5-(*n*-amyl)-resorcinol	11	0.58 ± 0.12

Examination of Table I reveals several interesting facts. In all cases, the secondary groups

(1) For previous paper, see Adams, Loewe, Theobald and Smith, THIS JOURNAL, **64**, 2653 (1942).

(2) Adams, Loewe, Jelinek and Wolff, *ibid.*, **63**, 1971 (1941).

(3) Russell, Todd, Wilkinson, Macdonald and Woolfe, *J. Chem. Soc.*, 826 (1941).

(4) Adams, Loewe, Smith and McPhee, THIS JOURNAL, **64**, 694 (1942).

(5) Wollner, Matchett, Levioe and Loewe, *ibid.*, **64**, 26 (1942).