temperature has risen to 35° and most of the solids have passed into solution. After two hours the temperature should be 45°, and some of the product will have begun to crystallize. The temperature should be 70° after twelve hours, and a large amount of the product then will have crystallized.

The alcohol is removed under diminished pressure, and the residue is dissolved in a liter of water. The solution is cooled and acidified with hydrochloric acid. The dry crude product weighs 21 g., contains the hydroxy acid as an impurity, and melts at 95–110°. After crystallization from 110 cc. of ligroin and 30 cc. of chloroform, it melts at 135–150°. It requires a second crystallization from 150 cc. of a 2:1 alcohol–water mixture and a third from 50 cc. of chloroform and 10 cc. of ligroin to raise the melting point to the constant value of 150°. The yield of pure product is 17.5 g.

It is not practical to remove the hydroxy acid from the cetyl and octadecyl derivatives by extraction with sodium bicarbonate solution on account of the troublesome emulsion. This is a suitable procedure for the lauryl derivative. However, one crystallization of the dry crude lauryl derivative from hot absolute alcohol gives a good product. In using the same slow heating process but double the quantity of alcohol the yield of the lauryl derivative drops from 82 to 68%.

5-Alkyl-5-(β-bromoethyl) Barbiturates.—In using the method of preparation previously reported, a difficulty arises in that the hydroxyethyl barbiturates do not dissolve in the fuming hydrobromic acid as in the case of the lower homologs. The following modification applied to the octadecyl derivative gave a yield of 70%.

5-Octadecyl-5-(β-hydroxyl)-barbituric acid (25 g.), chloroform (50 cc.), and 70% hydrobromic acid (100 cc.) are heated in a soda water bottle for four hours at 50–60°. The crude product weighs 27 g. and melts at 98–102°. After crystallizing once from dilute alcohol and twice from a 1:2 chloroform–ligroin mixture, there is obtained a pure product weighing 20 g. and melting at 104.5°.

5-ALKYL-5-(β-HYDROXYETHYL) BARBITURATES

Alkyl	M. p., °C.	Yield, %	Found N, %	Calcd. N, %
n-$C_{12}H_{25}$-	145	82	8.29	8.23
n-$C_{16}H_{33}$-	147	83	7.06	7.07
n-$C_{18}H_{37}$-	150	81	6.62	6.60

5-ALKYL-5-(β-BROMOETHYL) BARBITURATES

Alkyl	M. p., °C	Yield, %	Bromine, % Calcd.	Found
n-$C_{12}H_{22}$-	101.5		19.72	19.66
n-$C_{16}H_{33}$-	102.5	65	17.01	17.08
n-$C_{18}H_{37}$-	104.5	70	16.40	16.33

Summary

α-Alkyl-α-carbethoxy-γ-butyric lactones have been employed in the synthesis of barbiturates containing large radicals at position five.

NEWARK, DELAWARE RECEIVED JULY 22, 1941

[CONTRIBUTION FROM ALLERGEN INVESTIGATIONS, BUREAU OF AGRICULTURAL CHEMISTRY AND ENGINEERING, U. S. DEPARTMENT OF AGRICULTURE]

The Chemistry of Allergens. V. The Amino Acid Content of Active Protein and Polysaccharidic Protein Fractions from Cottonseed[1]

BY JOSEPH R. SPIES

This paper presents results of the determination of the amino acid content of allergenic fractions obtained in a previously described electrophoretic fractionation[1] of CS-1A.[2] CS-1A proved to be a mixture consisting of specifically active protein and compounds of this protein containing polysaccharidic carbohydrate in varying proportions. Fractions CS-51R, and CS-52R, which migrated toward the cathode, contained less than 1% carbohydrate while the anodic fraction CS-56H contained 37% carbohydrate. Quantitatively determined among the hydrolytic products of these fractions were cystine, histidine, arginine, lysine, glutamic acid, tyrosine, humin, and ammonia. Results are summarized in Table I.

Discussion

The protein component of CS-1A appears to be analogous to the "natural proteoses" from vegetable sources which Wells and Osborne studied.[3] These authors, however, did not determine the amino acid content of their proteoses.

On the basis of available analyses Mitchell and

(1) (a) Paper IV of this series, Spies, Bernton and Stevens, THIS JOURNAL, **63**, 2163 (1941). The cottonseed used in this and previous investigations in this Laboratory was choice quality, dehulled, American grown, seed of the Upland Group. This material should therefore be designated *Gossypium hirsutum* rather than *Gossypium herbaceum* as reported in Paper I of this series. S. C. Harland, "The Genetics of Cotton," Jonathan Cape, London (1939). (b) Not copyrighted.

(2) Spies, Coulson, Bernton and Stevens. THIS JOURNAL, **62**, 1420 (1940).

(3) Wells and Osborne, *J. Infectious Diseases*, **17**, 259 (1915). Their proteoses were apparently distinct from other reserve proteins of seeds, possessed strong anaphylactogenic activity and were stable to heating to 100°. These authors stated "They resemble highly soluble native proteins in their anaphylactic capacity and are probably quite as complex in their chemical constitution." The cottonseed allergen is, however, more diffusible than the proteoses of Wells and Osborne, indicating lower molecular weight. A paper describing antigenic properties of cottonseed allergenic fractions has appeared elsewhere; Coulson, Spies and Stevens, *J. Immunol.*, **41**, 375 (1941).

TABLE I

AMINO ACID CONTENT OF ALLERGENIC FRACTION FROM COTTONSEED (EXPRESSED AS PER CENT. OF THE TOTAL NITROGEN)

Nitrogen in the form of:	Fraction[a] CS-51R[b]	CS-52R[b]	CS-56H
Humin	0.1	0.1	1.1
Ammonia	15.0	14.9	9.9
Cystine[c]	4.5	4.9	4.3
Histidine[d]	0.1	0.1	..
Arginine[e]	32.8	33.2	29.5
Lysine[f]	3.8	1.6	2.0
Glutamic acid[g]	14.2	14.2	11.4
Tyrosine[h]	1.7	1.8	1.3
Monoamino fraction[i]	8.0	..	11.2
Dicarboxylic acid fraction[j]	3.5	..	7.4
Total	83.7	70.8	78.1

[a] Fractions CS-51R, CS-52R and CS-56H contained 19.8, 20.2, 11.6% nitrogen; 2.29, 2.24 and 2.04% sulfur; and 0.9, 0.9, and 36.6% carbohydrate, respectively. All analyses were on ash-water free basis. It is recognized that the values obtained for the amino acids determined by isolation procedures are minimal. [b] Fractions CS-51R and CS-52R are essentially equivalent to fraction CS-13A.[2] [c] Cystine determinations were made by Dr. W. C. Hess of Georgetown University. Protein was hydrolyzed with 20% hydrochloric acid both with and without titanium chloride and with constant boiling hydriodic acid. The methods of Sullivan and Okudu gave concordant results. Cystine in CS-51R and CS-52R accounted for all of their sulfur content, but cystine in CS-56H accounted for only 55% of the total sulfur. [d] Determined by the Kapeller–Adler method after silver salt separation. [e] These values were obtained by the flavianic acid method described by Vickery. Corresponding values for CS-51R, CS-52R and CS-56H were 30.2, 29.2 and 24.7, respectively. by the silver salt method. [f] Isolated as the picrate. [g] Isolated as the hydrochloride. [h] Determined by the Folin–Marenzi method using 100-mg. samples of protein, *J. Biol. Chem.*, **83**, 89 (1929). [i] This unidentified nitrogen was considered the monoamino acid fraction because the barium salt was soluble in alcohol. This value has been corrected for all of the tyrosine, although some tyrosine also occurs in the dicarboxylic acid fraction. [j] This unidentified nitrogen occurred in the filtrate after precipitation of glutamic acid hydrochloride. Analytically pure copper aspartate could not be obtained from this filtrate.

Hamilton[4] pointed out that proteins of nuts and certain oil-bearing seeds were uniformly rich in arginine (>20% of the total nitrogen) moderately rich in lysine and relatively poor in histidine. For the most part these arginine-rich proteins were globulins.[5] Although cottonseed allergenic proteins resemble these globulins in the relative proportions of their basic amino acid components they differ markedly in other properties. The clinical observation that many cottonseed sensitive patients are also sensitive to certain oil-bearing seeds and nuts has been frequently observed and previously recorded by Bowman and Walzer.[6] In general, allergens from the oil-bearing seeds and nuts are of highest potency of any from vegetable sources. A generalization concerning a correlation between composition and immunological properties is not justified by available evidence. However, the point seems worthy of mention that coexistent allergic sensitiveness to cottonseed, nuts and other oil-bearing seeds may be owing to proteins like 51-R of high arginine content.

Experimental[7]

Preparation of Allergenic Proteins.—Details of isolation and purification of the protein fractions, CS-51R and CS-52R and the polysaccharidic-protein fraction CS-56H, are given in Paper IV[1] of this series.

Methods Used in Determining Amino Acids.—Three-gram samples of protein (ash-water free) were hydrolyzed by refluxing for twenty-four hours with 75 ml. of constant boiling hydrochloric acid. The acid was removed from the hydrolyzate by vacuum distillation on a steam-bath. Hydrolyzate from a single 3-g. sample was used to determine humin, ammonia, histidine, arginine, lysine, glutamic acid, and the unidentified fraction of nitrogen in the dicarboxylic and monoamino acid groups.

The insoluble black material which remained when the residue from the hydrolyzate was dissolved in water was determined as humin.

Ammonia was determined by vacuum distillation, into standard acid of the hydrolyzate which was made alkaline with barium hydroxide. The temperature was kept below 35° during the distillation of the ammonia.

Basic amino acids were precipitated by phosphotungstic acid.[8] The Kossel and Kutscher silver salt method modified by Vickery and Block[9] was then used to separate the diamino acids.

Histidine was determined colorimetrically by the Kapeller-Adler method.[10]

Arginine from the silver salt separation was isolated as the monoflavianate.

Anal. Calcd. for $C_{16}H_{20}O_{16}N_6S$: N, 17.2; S, 6.56. Found[11]: N, 17.1 ± 0.2; S, 6.44 ± 0.07.

Arginine was also determined by the flavianic acid method described by Vickery,[12] using 250 to 500 mg. samples. *Anal.* Found[11]: N, 17.1 ± 0.1; S, 6.35 ± 0.09.

(4) Mitchell and Hamilton, "The Biochemistry of the Amino Acids," The Chemical Catalog Co., New York, N. Y., 1929, pp. 182–188.

(5) The pentose protein from cottonseed isolated by Jones and Csonka, *J. Biol. Chem.*, **64**, 673 (1925), appears to be an exception.

(6) Coca, Walzer and Thommen, "Asthma and Hay Fever in Theory and Practice," Charles C. Thomas, Baltimore, Md., 1931, p. 394. Several cottonseed sensitive patients used in this study also exhibited this type of multiple sensitivity.

(7) Microanalyses were made by E. J. Umberger.

(8) Van Slyke, *J. Biol. Chem.*, **10**, 15 (1911–1912); **22**, 281 (1915).

(9) Vickery and Block, *ibid.*, **93**, 109 (1931).

(10) Kapeller-Adler, *Biochem. Z.*, **264**, 131 (1933).

(11) These values are the average obtained for derivatives of the designated amino acids obtained from CS-51R, CS-52R and CS-56H.

(12) Vickery, *J. Biol. Chem.*, **132**, 325 (1940).

Lysine was isolated as the picrate which decomposed between 255 and 260°. *Anal.* Calcd. for $C_{12}H_{17}O_9N_5$: N, 18.7. Found[11]: N, 18.5 ± 0.1.

The dicarboxylic acids were separated from the monoamino acids by precipitation of their barium salts with alcohol.[13] Glutamic acid was then determined as the hydrochloride which decomposed sharply at 202–203°.

Anal. Calcd. for $C_5H_{10}O_4NCl$: N, 7.63. Found[11]: N, 7.65 ± 0.04.

(13) Jones and Moeller, *J. Biol. Chem.*, **79**, 429 (1928).

The Hopkins–Cole test for tryptophan was negative.

Summary

Cystine, histidine, arginine, lysine, tyrosine, glutamic acid, ammonia and humin were quantitatively determined in the previously described cottonseed allergenic fractions, CS-51R, CS-52R and CS-56H.

WASHINGTON, D. C. RECEIVED JULY 31, 1941

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY]

Researches on Thiazoles. XXIV. Some Interesting Exchange Reactions between 6-Alkoxy-7-nitrobenzothiazoles and Alcohols

BY H. HERBERT FOX[1] AND MARSTON TAYLOR BOGERT

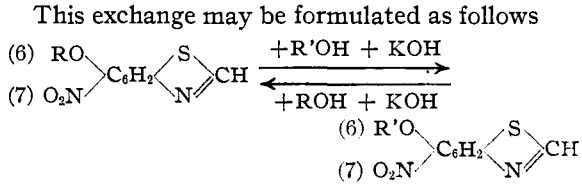
In the course of some experiments[2] to determine the location of the nitro group in what was subsequently proved to be the 6-methoxy-7-nitrobenzothiazole, attempts were made to break open the thiazole nucleus to the 2-amino-5-methoxy-6-nitrophenylmercaptan by fusion with potassium hydroxide,[3] and by the action of concentrated alcoholic potassium hydroxide solution.[4] In both cases, extensive decomposition resulted and no pure products could be isolated.

Recourse was had, therefore, to less drastic treatment, and an alcoholic solution of the nitro derivative, containing a small amount of potassium hydroxide, was heated for a short time on the steam-bath and the mixture, when cold, was poured into an excess of dilute hydrochloric acid. The product, on the basis of general appearance, solubilities, and melting point, obviously was neither the initial material nor the result of a scission of the thiazole nucleus, for it gave no tests for the presence of a primary amino, a mercapto, or a disulfide group, and an elementary analysis indicated an increase, and not a decrease, in carbon content. In short, the reaction had involved not the thiazole nucleus, but the methoxyl group, and the product was identified as the corresponding ethoxynitrobenzothiazole, evidently formed by an exchange between the methoxyl ions of the thiazole and the ethoxyl ions of the alcoholic alkali.

This reaction was reversible. When the ethoxy derivative so produced was subjected to the action of methanol containing a small quantity of potassium hydroxide, the original methoxy derivative was reformed.

The sensitivity of the alkoxyl group to alkali was shown by the saponification of the methoxy derivative with dilute aqueous alkali to the corresponding hydroxy derivative. All attempts to regenerate the alkoxy from the hydroxy derivative proved futile.

This exchange may be formulated as follows



In all cases, the other product of the reaction was the free hydroxy derivative, formed presumably by the direct action of the hydroxyl ions upon the ether.

As shown in Table A, such an exchange has been proved to take place with the following alcohols: methyl, ethyl, *n*- and *i*-propyl, *n*-butyl, β-phenylethyl, cyclohexyl, glycol, and ethanolamine. Glycol reacted with one and not with two moles of the thiazole, to give the β-hydroxyethyl, and not the dithiazylethylene derivative. Interestingly, the exchange reaction with ethanolamine did not require the addition of any alkali, the amino group apparently functioning similarly.

Isopropyl alcohol underwent the reaction more easily than the normal alcohol. It will be seen also from the table that the melting point of the ethers falls with increase in the molecular weight of the hydrocarbon radical, and that the isopropyl melts lower than the corresponding *n*-propyl

(1) Present address: Hoffmann-LaRoche Co., Nutley, N. J.
(2) Fox and Bogert, THIS JOURNAL, **61**, 2013 (1939).
(3) Hofmann, *Ber.*, **13**, 18 (1880).
(4) Mylius, *Chem. Zentr.*, **54**, 819 (1883).