The salts may be identified by their melting points and also by titrating a weighed portion of the salt with 0.01 *N* sodium hydroxide, using phenolphthalein as an indicator. The corrected melting points and molecular weights of the salts are found in Table I. An analysis for sulfur on the salt of methane sulfonic acid gave 15.81%; calculated 15.72%. Since the observed molecular weights checked well with the calculated values, it was deemed unnecessary to run sulfur analyses on the entire list. Mixed melting points were run on the salts from butane sulfonic acid-1 through octane sulfonic acid-1. Where the two acids differed by one methylene group the depression was found to be about three degrees. Where the acids differed by two methylene groups the depression was about fifteen degrees.

TABLE I

MELTING POINTS OF THE PHENYLHYDRAZINE SALTS OF CERTAIN ALIPHATIC SULFONIC ACIDS

Phenylhydrazonium	M. p., °C. (corr.)	Mol. wt. Obsd.	Mol. wt. Calcd.
Methane sulfonate	193.5–194 (dec.)	206.5	204.0
Ethane sulfonate	182.8	218.0	218.2
Propane sulfonate-1	204.5 (dec.)	233.0	232.1
Butane sulfonate-1	114–115	244.5	246.2
Pentane sulfonate-1	108–108.2	258.0	260.2
Hexane sulfonate-1	101–101.6	275.0	274.2
Heptane sulfonate-1	100–100.5	292.5	288.2
Octane sulfonate-1	90–90.5	303.3	302.2

The salts may be prepared on a much smaller scale by using 0.001 mole of the barium salt and corresponding quantities of the other reagents. This will easily give enough material for melting points. If the molecular weight is to be determined, it is suggested that the larger quantities be used.

Summary

1. Phenylhydrazine forms crystalline salts with normal aliphatic sulfonic acids.

2. The salts may be identified by melting point and also by titrating with 0.01 *N* sodium hydroxide to obtain the molecular weight, thus serving as a check on the compound.

3. The constants for the salts of the sulfonic acids from methyl through *n*-octyl are given.

4. The reagent is easily available, the method is simple and the melting points are well defined.

CHAPEL HILL, N. C. RECEIVED SEPTEMBER 23, 1937

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Natural and Synthetic Rubber. XVIII. The Protein from Natural Rubber and its Amino Acid Constituents

BY THOMAS MIDGLEY, JR., ALBERT L. HENNE AND MARY W. RENOLL

The material remaining after the extraction of natural rubber with organic solvents has been known to contain nitrogen, and to exhibit some protein characteristics.[1,2] The numerous communications on the subject have been compiled and sifted adequately.[3] Only one investigation has been concerned with the amino acids resulting from the hydrolysis of the protein material.[4]

The purpose of the present work was to isolate a product which would be as nearly pure natural rubber protein as possible, to analyze it and to separate and identify the individual amino acids resulting from its hydrolysis.

The starting material was the nitrogen containing residue obtained in the preparation of pure rubber hydrocarbon by fractional precipitation from a mixture of benzene and alcohol.[5] This was a tough brown mass, entirely free of resins, but still containing a large amount of rubber, all the mineral impurities, some sugars and the protein. Depending upon the extent of its treatment, it titrated from 3.5 to 4.2% nitrogen. This material was allowed to swell in a large quantity of xylene kept at 100° in a water-bath and never stirred. Every twenty-four hours, the liquid was decanted with the least possible stirring, and fresh or recovered xylene poured on the rubber to take its place. After a few days, the material became quite crumby and progressively lost its tackiness. After a week, xylene did not remove any more rubber. The material was then boiled with xylene to extract the last traces of rubber. The crude protein separated easily from the xylene. When boiling or stirring was resorted to too early, the protein dispersed in the rubber solution, and considerable loss occurred during decantation; moreover, separation was rendered quite incomplete and difficult to handle.

After removal of the xylene by means of boiling benzene, the protein was placed in an evaporating dish to dry in a current of air; the dry material was a friable, fluffy, tan powder, whose nitrogen content varied from 5 to 8%, and ash content from 15 to 35%. The next step consisted in electrodialyzing at 120 volts through cellophane; this removed the inorganic impurities and carbohydrates.

The purified protein, dried in a vacuum oven at 70° for six hours was a brown, crumby mass; it amounted to about 45% of the material undergoing electrodialysis. It was analyzed for carbon and hydrogen by combustion, and for nitrogen by Kjeldahl, the final result being on an ash-free, dry basis: 57.68% C, 7.54% H, 12.52% N and 22.28% O (computed by difference), corresponding to an

(1) Weber, *J. Soc. Chem. Ind.*, **19**, 215 (1900).
(2) Spence, *India Rubber J.*, **2**, 766 (1907).
(3) Dinsmore, *Ind. Eng. Chem.*, **18**, 1140 (1926).
(4) Belgrave, *Malayan Agr. J.*, **13**, 154 (1925).
(5) Midgley, Henne and Renoll, THIS JOURNAL, **53**, 2733 (1931).

empirical formula: $C_{5.35}H_{8.36}N_{1.0}O_{1.55}$, or an approximate $(C_{10}H_{16}N_2O_3)_x$.

This value for nitrogen is confirmed by the analysis of several purified samples from which prolonged extraction failed to remove any more rubber. These, when corrected for ash content, gave by titration 12% of nitrogen. A critical examination of the work of previous investigators also indicates 12% as their best value for nitrogen. These observations are stressed because of the still prevalent practice, particularly in stating the results of the analysis of rubber, of multiplying the nitrogen content by 6.25 to compute the protein content of the material, which of course arbitrarily assumes the nitrogen content of a protein to be 16%.

Hydrolysis of the purified protein material was obtained by treating 11 g. of it with 100 cc. of dilute sulfuric acid (40 cc. of concd. acid to 140 cc. of water), boiling under reflux. The hydrolyzate was filtered to remove 3.18 g. of humin, whose nitrogen content was found to be 0.7%. This humin was treated with benzene, which removed about one-half of it. From the benzene solution, alcohol precipitated a substance which had all the appearances of rubber. Making the assumption that this material was rubber, it is possible to recompute the analysis result of the protein, and to find that the nitrogen content would be somewhere between 15 and 16%, which is the nitrogen content generally expected in a protein. It should, however, be repeated that removal of the last rubber by solvents was found impossible before completion of the hydrolysis.

The hydrolyzate was separated into its constituents by the carbamate method,[6] as more recently improved.[7] The dibasic amino acids were isolated and separated by the method of Block.[8]

When needed, final identification was obtained by preparing crystalline derivatives, according to the procedure of Crosby and Kirk[9] and comparing them with the published photomicrographs. The picrates were used to characterize glycine, leucine, and proline, and the flavianate to identify aspartic acid and leucine.

The amino acids found and definitely identified were: glycine, aspartic acid, leucine, proline, arginine, histidine, lysine and representative of the group comprising alanine, phenylalanine, hydroxyproline and serine. This group was not investigated further. The amino acids which were definitely absent included cystine, tyrosine and glutamic acid.

Summary

The protein constituent of rubber has been extracted from the natural rubber by removal of the rubber hydrocarbon with solvents, followed by electrodialysis of the residue. This material has been analyzed, subjected to hydrolysis, and its amino acid constituents have been separated and identified.

(6) Kingston and Schryver, *Biochem. J.*, **18** [5], 1070 (1924).
(7) Caldwell and Rose, *J. Biol. Chem.*, **107**, 45 (1934).
(8) Block, *ibid.*, **106**, 457 (1934).
(9) Crosby and Kirk, *Mikrochemie*, **18**, 137 (1935).

THE MIDGLEY FOUNDATION
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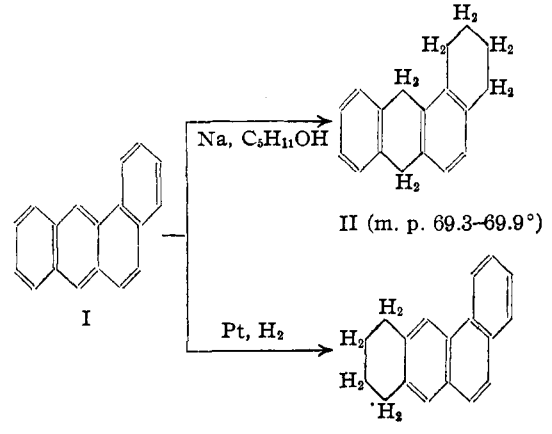
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Reduction and Hydrogenation of Compounds of the 1,2-Benzanthracene Series

BY LOUIS F. FIESER AND E. B. HERSHBERG[1]

The investigation of the reduction and hydrogenation of carcinogenic hydrocarbons of the 1,2-benzanthracene series is of interest both because of the possibility of obtaining in this way new compounds of value in further defining the limits of carcinogenic activity and because a knowledge of the center, or centers, of chemical reactivity in the molecule of an active carcinogen may be of value in understanding the biological actions of the compound. The recent development of a convenient synthesis of 1′,2′,3′,4′-tetrahydro-1,2-benz-10-anthrone[2] (VI, below) made available a starting material for the preparation of new reference compounds of known structure and provided the occasion for the present work.

With 1,2-benzanthracene itself we have been able to establish that reduction with sodium and amyl alcohol, and by low pressure hydrogenation, proceeds as indicated in formulas II and III, respectively. The first reaction gave a nicely



(1) Lilly Research Fellow.
(2) Fieser and Hershberg, THIS JOURNAL, **59**, 2331 (1937).