STUDIES ON "SILAJIT" (ASPHALT)

Part I.—Composition of the Mineral and Proteinous Matter

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(Received August, 15 1964)

"Silajit" (asphalt) has been studied with respect to its proteinous and mineral matter. The quality as well as quantity of its amino acids is indicative of its animal origin.

Introduction

'Silajit' or Momiae, a dark, sticky, bituminous substance is obtained in mountainous regions of Asia. In the Indo-Pakistan Sub-continent it is collected during the months of May to July, when the weather is very hot. The use of the drug is lengendary in the East. Particularly it is an important drug of the Hindu and Muslim materia medicas. It has been used for various diseases especially those of the geneto-urinary system and against diabetes, gallstone, renal stone, anuria, anasarca tuberculosis, neurasthenia, eczema etc.[1–3]

From time to time attempts have been made to find out the chemical composition and physiological effects of the drug. Chopra *et al.*[3] reported that the drug contains benzoic acid (18.58%) hippuric acid (6.13%), fatty acids (1.36%), resin and waxy matter (2.44%), gums (17.32%), albuminoids (16.12%) and mineral matter (34.95%).

The mineral matter has been shown to contain silica (4.6%), iron (0.51%), alumina (2.26%), lime (6.83%), magnesia (1.29%) potash (4.6%) sulphuric acid (0.64%) phosphoric acid (0.28%) chloride (0.26%) and nitrogen (3.64%). Detailed studies on the composition of the various organic fractions, however, have not so far been reported.

Therapeutically, the drug was shown to have antidiabetic action [4] and most of its medicinal effects were attributed to benzoic acid and benzoates which are present in the drug in a fairly large amount.

An important question which so far has remained unanswered in the investigations on this drug pertains to its origin. Some insight into this question can probably be gained by a thorough examination of its constituents. In the present studies, therefore, the drug has been investigated with respect to the constituents of the following fractions of the drug: (1) mineral matter, (2) proteinous matter (3) fatty acids (4) gums and (5) resin and waxy matter. This communication deals with the work on (1) mineral matter and (2) proteins. The other fractions would be dealt with in a subsequent report.

It is difficult to obtain silajit in a pure state as natural silajit contains clay, dust, etc., and the material available in the market is often adulterated with various foreign matter e.g., burnt sugar, coal-tar, pitch, etc. The silajit sample for the present investigations was especially obtained through the courtesy of Shahzada Hissamul Mulk, Governor of Drosh, Chitral State.

Experimental

Isolation of Proteins from Silajit[3]

20 g. of silajit were extracted by hot percolation with 80 percent ethanol till the percolate was colourless. The alcohol was distilled out and the extract dried on a water bath and then under reduced pressure. The dried extract was dissolved in hot water, filtered and the filtrate shaken with chloroform, and then repeatedly with ethyl acetate to remove benzoic acid, hippuric acid and an oily substance. The aqueous solution was next heated on a water bath to remove traces of ethyl acetate, cooled and acidified with dilute hydrochloric acid, when a turbidity appeared at once. The turbid solution was repeatedly extracted with chloroform till the solution became clear. The solution was finally evaporated to dryness on a water bath. The nitrogen content of the residue obtained was 2.15 percent as determined by the Markham micro method.[5]

Acid Hydrolysis of the Proteins and Preparation of Hydrolyzate.[6]—0.7 g. of the proteins were hydrolysed with 20 ml. of 6N hydrochloric acid for 24 hours. Temperature was adjusted to keep acidic mixture just near boiling. The solution was filtered through sintered funnel and the residue washed with distilled water. The combined filtrate and

the washings were evaporated to dryness on a water bath. Water was once more added and the contents heated again to dryness. The procedure was repeated till the solution was free from hydrochloric acid. Last traces of the acid were removed by keeping the dried mass overnight over soda lime in a vacuum desiccator. The hydrolyzate was taken up in exactly 40 ml. of 10 percent *iso*-propanol.

Alkali Hydrolysis of the Proteins for Tryptophan.[7]—0.5 g. of the proteins were hydrolysed for 20 hours with 10 ml. of 14 percent barium hydroxide solution at 120-125°. The excess of barium hydroxide was removed by the addition of sulphuric acid and the solution cleared by centrifuging. The solution was concentrated to a small volume under reduced pressure and then evaporated to dryness in a desiccator over calcium chloride. The residue was taken in 1 ml. of 10 percent *iso*-propanol and then examined chromatographically for tryptophan which was found absent.

Qualitative Analysis for Amino Acids in the Protein Hydrolyzate.—For the identification of the amino acids in the hydrolyzate, uni-dimensional descending chromatographic technique[8] was employed as follows:

A sheet of Whatman paper No. 1 (18″×11″) was cut and pencil line was drawn across the strip about 3 inches from one end. The silajit protein hydrolyzate as well as solutions of twenty-three known standard amino acids (10 mg. of each amino acid per ml.) were applied on the paper, from the tip of a capillary tube. The hydrolyzate as applied at the centre of the line and the amino acid solutions on either side at places 1 inch apart. The chromatographic trough and the paper were then transferred to an all-glass chamber and the trough filled with the solvent. When the solvent had run a sufficient distance (16″), the paper was dried and sprayed with a solution of ninhydrin (0.25% w/v in acetone) and dried. Finally it was heated at 80°C. for 5 minutes. The bands were outlined immediately in pencil as fading of the ninhydrin colour takes place after some time. Eight clear separate spots were obtained which matched with the standard specimens of glycine, hydroxy proline, alanine, threonine, proline, tyrosine, valine and isoleucine. These were further confirmed from their Rf values reported in Table 1.

Quantitative Estimation of the Amino Acids.—Standard graphs of the amino acids constituting the silajit proteins were prepared by using pure and dry specimens (E. Merk). For each amino acid spots of varying concentrations i.e. 10,20, 30,40,50,60,70, 80 and 100μg were applied on the filter paper in 0.01 ml. quantity using a calibrated micropipette and chromatographed as described under the Qualitative Analysis. The paper was dried and uniformly sprayed with ninhydrin (0.5% w/v in acetone). The spots were cut, and the cuts were extracted with 4 ml. of 75% ethanol containing 0.5 mg. of copper sulphate ($CuSO_4.5H_2O$).[9] The ethanolic extract in each case was made up to 10 ml. with 75% ethanol in a volumetric flask. The percentage transmissions of the solutions were read at 540 mμ on a Beckman spectrophotometer model, D.B. Percentage transmission versus concentration in μg graphs were drawn which showed a linear relationship.

TABLE 1.—RF VALUES OF AMINO ACIDS WITH *n*-BUTANOL: ACETIC ACID: WATER 25:6:25, RESPECTIVELY.

	Standard amino acid	Silajit hydrolyzate amino acid
1. Glycine	0.183	0.181
2. Hydroxy proline	0.250	0.244
3. Threonine	0.300	0.293
4. Alanine	0.358	0.342
5. Proline	0.410	0.397
6. Tyrosine	0.473	0.475
7. Valine	0.547	0.545
8. Isoleucine	0.660	0.650

0.01 ml of the silajit hydrolyzate was applied on the paper and chromatographed, developed and eluted as above. The concentration of each amino acid was read off from the standard graphs against the observed transmission in each case. The quantities of the amino acids are presented in Table 2.

Mineral Matter.—The constituents of the silajit ash were determined both by spectroscopic as well as X-ray analysis and the results are represented here.

CONSTITUENTS OF SILAJIT ASH

CaO,40; $MgO + Mg(OH)_2$,30; $NaOH$,10; KOH,10; Fe_2O_3,4; SnO,2; SiO_2,2; Cu, traces.

Discussion

The analysis of the mineral matter of silajit is not particularly indicative of the origin of this drug. Most of this matter could as well have been incorporated in the drug from the rock from which it exudes and therefore, to base any conclusion on this analysis is rather hazardous.

TABLE 2.

Spot No.	Amino acids	Percentage transmission in 0.01 ml. hydrolyzate 1	2	3	Mean	Micro grams of amino acid in 0.01 ml. of hydrolyzate	Amount in 10 g. proteins mg.	Amount in 100 g. proteins g.
1.	Glycine ..	77.0	77.5	76.5	77.0	35.0	200.0	20.0
2.	Hydroxy proline ..	85.0	86.0	85.0	85.3	15.5	89.0	8.9
3.	Threonine ..	85.0	86.0	85.0	85.3	31.05	178.0	17.8
4.	Alanine ..	79.0	79.0	79.0	79.0	23.0	131.0	13.1
5.	Proline ..	87.0	86.5	87.0	86.8	40.0	230.0	23.0
6.	Tyrosine ..	85.0	85.5	85.0	85.16	2.5	14.3	1.4
7.	Valine ..	82.0	82.5	82.0	82.16	1.5	8.6	0.86
8.	Isoleucine ..	83.0	83.5	83.0	83.16	10.01	5.7	0.57

It is, however, well-known that the organic fractions such as proteins, lipids, etc. are typical of a given species. The protein composition of vegetable matter differs widely from that of the proteins of animals. There are certain amino acids which would be present in one kingdom while they would either be present in minute quantity or absent altogether in the other. This patent difference, therefore, constitutes the grounds on which conclusion regarding the origin of silajit has been based.

Amino acid contents of the proteinous matter of silajit are shown in Table 2. It is seen that the matter has high glycine, proline, threonine and hydroxy proline contents. From the studies of various plant and animal proteins [10–11] it can be concluded that silajit proteins resemble more with animal than with plant proteins and still more they match with keratins and skin proteins. The unusual feature of the keratins and skin proteins is that they are composed of high proportions of glycine, proline and hydroxy proline. The two last named amino acids occur rarely in other proteins.[12] It can, therefore, be concluded safely that the proteins of silajit belong to an animal origin. In the previous work it was reported by Chopra *et al.*[3] that silajitcould be a compact mass of vegetable organic matter as vegetable fibres were found to be present in the drug on microscopic examination. But our investigations do not support this hypothesis. It is likely that this drug might have been contaminated with vegetable fibres from grass, leaves, roots, etc.

n-butanol-acetic acid-water[13] mixture in the ratio of 25:6:25 gave well defined spots with the hydrochloric acid hydrolyzate of the silajit proteins. Best resolution was achieved when the temperature of chromatographic tank was maintained at 30°C. throughout.

Acknowledgement.—The help of Dr. M.M. Qurashi, Chief Scientist, Ministry of Defence, for the analysis of the mineral matter, is acknowledged.

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