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Salvia Carnosa (Dougl.). I—A Phytochemical Study*

By Allen I. White† and Glenn L. Jenkins‡

Salvia carnosa (Dougl.) is a North American plant of the Labiatae family found on the plains and slopes west of the Rocky Mountains from eastern Washington as far south as Nevada and California. It is a leafy, low, broad shrub, three-fourths to two and one-half feet high, bearing long spatulate or obovate, obtuse or retuse leaves. The bracts and upper floral leaves are tinged with rose or purple and the corolla is a deep blue. It is commonly called Purple Sage.

Taxonomists who questioned the classification of this plant in the genus *Salvia* have given it other botanical names (*Audibertia incana, Ramona incana*) but present-day taxonomists apply to it the name originally given by Douglas, that is, *Salvia carnosa.*

Attention was first directed to this plant because of its reputed use by the Indians as a medicinal herb in the treatment of colds, pneumonia, headache, stomachache, and for eyewashes (1). Heretofore, *Salvia carnosa* has received no attention as a possible source of drug material and no investigation of its character or chemistry has been found described in the literature.

The plant material used in this investigation was collected in the vicinity of Reno, Nevada, during the growing season of 1938, and was identified by W. Andrew Archer of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, United States Department of Agriculture. The collection was divided into three portions. The first consisted of the entire overground plant, and preliminary investigation indicated that 50% alcoholic extracts possessed possible bacteriocidal activity.[1] This material was used in the general phytochemical studies. A second sample which also consisted of the entire overground plant did not possess appreciable bacteriological activity. It was not further

* Abstracted from a part of the thesis presented to the Graduate Faculty of the University of Minnesota by Allen I. White in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

This is a report in a coöperative research project connected with the Indian Medicinal Plant study of the Bureau of Plant Industry, United States Department of Agriculture.

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† Instructor, School of Pharmacy, State College of Washington. Former Bureau of Plant Industry Agent.

‡ Dean, School of Pharmacy, Purdue University. Former Professor of Pharmaceutical Chemistry, College of Pharmacy, University of Minnesota.

[1] The bacteriological tests were conducted by Dr. R. N. Bieter, Dr. W. P. Larson, Charles H. Drake and J. Gordon Beaton of the School of Medicine, University of Minnesota.

used in this investigation. The third sample consisted entirely of leaves and was used as a source of volatile oil. Investigation showed that the oil did not possess the characteristic bacteriological action of the extracts of the first sample. The object of this investigation was to make a preliminary phytochemical study of the whole plant material and especially to isolate and investigate the chemistry of the constituent responsible for the bacteriological activity of the extracts and the purported medicinal action obtained by the Indians.

EXPERIMENTAL

The air-dried, dirt-free material was ground to about a No. 20 powder and well mixed. Using U. S. P. methods, it was found to contain 5.14 per cent total ash and 7.92 per cent of moisture.

Preliminary Examination.—The amount of volatile oil present in each of the three samples submitted for investigation was determined by the Clevenger method (2). The first overground sample contained 0.06%, the second contained 0.02%, and the leaves contained 3.46%.

One hundred grams of the powdered material were subjected to successive extractions with various solvents in a Soxhlet apparatus. The per cent of extractive was determined by evaporation of the solvent and drying of the residue at 100° C.

Solvent	Per Cent
Petroleum benzin (b. p. 60–70° C.)	2.40
Diethyl ether	0.70
Chloroform	0.70
Ethanol	4.50
Ethyl acetate	0.05
Water	3.80
Total per cent of extractive	12.15

The petroleum benzin extract was a reddish brown resinous residue. When it was dissolved in the smallest amount of petroleum benzin possible and cooled in a refrigerator for several hours, 0.45 Gm. of a light green wax precipitated out. It was purified by dissolving in a small amount of hot methanol and then cooling the solution and collecting the resulting amorphous precipitate. The diethyl ether, chloroform, alcohol and ethyl acetate extracts were brown, resinous residues. The water extract was brown in color, had a hard and brittle consistency, was practically odorless and had a slightly sweet taste.

Maceration and extraction of a portion of the plant material with Prollius' fluid failed to yield alkaloids as indicated by tests with the usual alkaloidal reagents. Examination for glycosides by a modified Stas-Otto method (3) gave negative results and glycosides were assumed to be absent.

LARGE SCALE EXTRACTION

Four kilograms of the ground material were extracted by placing 1 Kg. at a time in a large glass percolator and extracting in the usual manner after a 24-hour maceration period with 50% aqueous ethanol. The procedure was allowed to continue until the percolate was nearly clear and no longer gave a test with ferric chloride test solution. The total extract from 1 Kg. of material amounted to about six liters.

Isolation of Crystals.—The extract so obtained was placed in a refrigerator and allowed to stand for two weeks. At the end of this period considerable crystalline material had collected and was removed by filtration. The filtrate was then replaced in the refrigerator and after standing for another two weeks, a second crop of crystals was collected. This procedure was repeated as often as necessary until no more crystalline material collected. Five or six collections were usually made. It was found by experiment that extreme cold or removal of ethanol would not facilitate the collection of the crystals without serious contamination. The crystalline material so obtained was readily purified by refluxing with charcoal in ethanol and after filtration, adding hot water to the hot solution to the point of incipient cloudiness and then allowing to cool gradually. Five recrystallizations from dilute alcohol produced clean, white, well-defined crystals. After drying for 24 hours over sulfuric acid in a desiccator, the total yield from 4 Kg. amounted to 25.2 Gm. or 0.63% of the total plant material. The material so obtained has been given the name *"Carnosol."*

Treatment of Extract with Acid.—The slow separation of carnosol from 50% ethanol extracts suggested that it might be the product of enzyme action on a glycoside. To study the effect of acid upon the production of carnosol, sufficient hydrochloric acid to make a 0.5% solution was added to one liter of extract obtained in the usual way. This extract was shaken thoroughly and placed in a refrigerator with five other liters of extract. While the acid extract became slightly cloudy and deposited more impurities, no appreciable increase in rate of separation or amount of carnosol over the neutral extracts could be detected.

Physical Examination of Crystals.—Highly purified carnosol consists of white, odorless long needles which upon ignition burn without leaving a residue. After making sure the crystals were perfectly dry and free of any possible solvent of crystallization by drying for two hours at 78° C. and 2 mm. of pressure in an Abderhalden Drying Pistol containing phosphoric anhydride, the following physical constants were determined:

1. *Melting point:* The correct melting point of the compound was difficult to ascertain because it began to turn brown at about 190° C. and gradually softened until it melted with decomposition at 217° C. (uncorr.). When the melting-point tube was immersed in a bath at 215° C. and heated at the

rate of 1° every 20 seconds, the melting point was found to be 219.5° C. (uncorr.) with decomposition.

2. Specific rotation: When dissolved in dealdehyded ethanol and using a sodium flame and a 1-dm. tube in an ordinary polarimeter, the specific rotation of carnosol was found to be −66.00°.

3. Solubility: Carnosol is soluble in ethanol, ether, methanol and chloroform, and is slightly soluble in petroleum benzin. It is soluble with coloration in dilute alkali; alkaline solutions undergo a change from a beginning light orange-red to a deep brown to blue-black. The crystals were soluble also in concentrated sulfuric acid, producing a yellow color. Carnosol is insoluble in water, dilute hydrochloric acid and 5% sodium bicarbonate solution.

Examination of 50% Ethanol Extract after Removal of Carnosol.—When no more crystalline material could be isolated from the 50% ethanol extract, the solvent was removed by distillation under diminished pressure. The residue consisted of a dark brown resinous mass from which the water-soluble material was removed by the following method: To the residue in a 2-L. beaker on a steam bath were added 500 cc. of boiling water and the mixture stirred mechanically for one-half hour. The mixture was then allowed to cool and the insoluble material settled to the bottom of the beaker. The water-soluble fraction was then decanted off through a filter paper. This procedure was repeated two more times with 500-cc. portions of boiling water. The third extraction was very little colored. The water was removed from the combined aqueous extracts by distillation under diminished pressure. The light brown residue had a sweet taste and a very slight aromatic odor. The presence of carbohydrates in this extract was confirmed by the Molisch test (4). A small portion of the aqueous extract quickly reduced Fehling's solution, indicating the presence of reducing sugars. When a small amount of the extract was dissolved in water and a drop of ferric chloride test solution added, a deep blue-green color resulted which was probably due to the presence of tannins since bacteriological tests indicated that the residue was inactive. No further examination of this extract was made.

The portion of the 50% ethanol extract insoluble in water was a dark brown resin that possessed an aromatic odor. It was completely soluble in 95% ethanol. Since bacteriological tests indicated that the residue was inactive, no further examination was made of it.

Examination of Marc of 50% Ethanol Extract.—The marc remaining after the 50% ethanol extractive had been removed was subjected to the following examination: The material was packed in a large Soxhlet apparatus and extracted with petroleum benzin. The light green extract obtained deposited upon cooling a green wax similar to the wax isolated from the petroleum benzin extract mentioned in the discussion of extraction with successive solvents. Bacteriological tests showed the wax to be inactive. Very little other material was present in the petroleum benzin extract and it was not subjected to further observation.

The marc remaining after the extraction with petroleum benzin was then subjected to extraction with diethyl ether. The ether was removed by distillation and a small amount of a dark brown resin resulted. Bacteriological tests indicated that the extract was inactive. No crystalline material was obtained from it and it was not further examined. The total amount of resin extracted by 50% ethanol and by ether amounted to 6.3% of the crude plant material.

VOLATILE OIL

Four and seventy-five hundredths kilograms of unground leaves were placed in a large still and steam distilled for six hours; 155 cc. of a volatile oil were collected from the distillate by mechanical separation and another 10 cc. were recovered from the aqueous portion by extracting it, after salting out, with petroleum benzin. The yield, weight to volume, was 3.46 per cent. Rectification by steam distillation produced 140 cc. of a clear, nearly colorless oil having an odor slightly resembling that of oil of eucalyptus. The rectified oil was dried by allowing it to stand over anhydrous sodium sulfate for 72 hours.

Specific Gravity.—The specific gravity as determined in a 10-cc. pycnometer fitted with a thermometer which dipped well into the liquid was 0.9209.

Specific Rotation.—The specific rotation of the volatile oil as determined in a polarimeter using a 1-dm. tube at 25° C. was +2.83°.

Refractive Index.—Using an Abbé refractometer, the average of five readings at room temperature was 1.4705.

Solubility in Alcohol at 25° C.—Five-tenth cubic centimeter of the volatile oil was placed in each of several graduated cylinders and alcohol of varying percentage strengths by volume was added until solution was just effected. The solubility is expressed as one volume of oil soluble or insoluble in a given volume of alcohol.

Solvent	Solubility
95% alcohol	1 in 1
85% alcohol	1 in 1
80% alcohol	1 in 12
70% alcohol	1 in 12 insoluble

The 1 in 12 solution of 80% alcohol is just slightly acid to litmus.

Saponification Value.—This value was determined according to the method given in the U. S. P. XI (5). Sample A: 48.14; Sample B: 48.41. Average saponification value: 48.27.

Acid Value.—Using the method given in the U. S. P. XI (6), the acid value found was 1.62.

Ester Value.—The ester value as determined by difference between the saponification value and the acid value was 46.65.

Acetyl Value.—For this determination, the method suggested by Allan (7) was used. The total acetyl

Table I.—Volatile Oil of *Salvia carnosa* (Dougl.) Fractionation

Fraction	Cc.	Boiling Point, °C. At 4 Mm. Pressure	Boiling Point, °C. At 730 Mm. Pressure	Refractive Index	Specific Gravity, 25° C.
1	18.6	40– 49	169–170	1.4596	0.9001
2	3.3	58– 65	177–181	1.4611	0.9169
3	7.5	80– 85	195–197	1.4708	0.9437
4	4.0	90– 95	Decomposition	1.4750	0.9460
5	3.5	97–100	Decomposition	1.4828	0.9362
6	2.5	102–110	Decomposition	1.4903	0.9229
7	4.0	113–123	Decomposition	1.4952	0.9282
Residue	6.6			1.5142	

value was calculated to be 89.20 and the actual acetyl value determined by subtracting the ester value from the total acetyl value was found to be 42.55.

Determination of Phenols.—The method suggested by Allan (7) was used for this determination. The volume of unabsorbed oil from a 5-cc. sample amounted to 4.75 cc., showing 0.25 cc. or 5% of the oil had been absorbed. Since an alcoholic solution of the volatile oil gave no color reaction with ferric chloride test solution, it is probable that the value obtained was due to the solubility of some ingredients other than phenols.

Investigation for Aldehydes and Ketones.—An unsuccessful attempt to obtain a solid derivative when the volatile oil was treated with hydroxylamine hydrochloride indicated the absence of aldehydes and ketones.

Fractionation of Volatile Oil.—Fifty cubic centimeters of the oil were placed in a 250-cc. flask fitted with a Vigreux fractionating arm, water condenser and a graduated vacuum adapter. A diminished pressure of 4 mm. of mercury was furnished from a Hy-vac pump and heat was applied from an oil bath. Table I shows the result of the fractionation and certain physical constants of each fraction.

DISCUSSION

In the experimental part of this paper, it was pointed out that the slow separation of carnosol from 50% ethanol extracts suggested the presence of a glycoside which upon enzyme hydrolysis yielded the crystalline product. While the presence of a glycoside could not be demonstrated by the Stas-Otto method, and the addition of acid to the extract did not increase the ordinary rate and amount of production of carnosol as might be expected if the production were dependent upon the hydrolysis of a glycoside, this is not sufficient evidence to disprove the glycoside theory. Many glycosides are soluble in 50% ethanol and the presence of a suitable hydrolyzing enzyme in such an extract is possible. However, enzymes are often inactivated by ethanol in concentration of 40–50% and higher. The possibility of a glycoside hydrolyzing during extraction must also be considered.

Since one sample of *Salvia carnosa* (Dougl.) which consisted of the entire overground plant yielded carnosol while another sample which also consisted of the overground plant did not, it appears that this compound may be formed at some particular stage in the growth of the plant. Further, since the sample which consisted of the leaves only was not a good source of the crystalline compound but was rich in volatile oil, while the overground samples did not contain an appreciable amount of volatile oil, it is possible that the crystalline compound is localized in the stems of *Salvia carnosa* and the volatile oil is localized in the leaves. These differences may also be due to varying local climate and soil conditions. Conclusive evidence in this matter can be derived only by further examination of the plant collected at known dates, during certain growth periods and at known places.

SUMMARY AND CONCLUSIONS

1. The source and a description of *Salvia carnosa* (Dougl.) have been given.

2. The total ash and moisture content of the plant have been determined.

3. The amount and type of extractives yielded to various solvents on successive extractions have been determined.

4. Qualitative tests indicate that the plant contains carbohydrates, including reducing sugars, and tannins, but that it does not contain alkaloids.

5. Examination by the modified Stas-Otto method did not show the presence of a glycoside, but the presence of one is held possible.

6. A new crystalline compound possessing possible bacteriocidal activity has been

isolated and some of its physical properties determined. It has been given the name "Carnosol."

7. A wax and a resin have been isolated from extractives of the plant.

8. A volatile oil has been isolated by steam distillation of the leaves of *Salvia carnosa* (Dougl.). The usual physical and chemical constants have been determined and the oil separated by fractional distillation into eight fractions.

9. It has been established that the three lots of *Salvia carnosa* (Dougl.) examined differed greatly in their content of volatile oil and carnosol.

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(4) Mulliken, "Identification of Pure Organic Compounds" (1911), John Wiley and Sons, New York, N. Y., Vol. I, p. 26.

(5) "United States Pharmacopœia XI" (1936), p. 445.

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Salvia Carnosa (Dougl.). II—Carnosol*

By Allen I. White† and Glenn L. Jenkins‡

In a previous paper (1) the authors reported the isolation of a new crystalline compound from *Salvia carnosa* (Dougl.). This compound, called carnosol, crystallizes in white, odorless, long needles and has a melting point of 219.5° C. (uncorr.) with decomposition. It has a specific rotation of −66.00° and is soluble in ethanol, methanol, ether and chloroform, and is slightly soluble in petroleum benzin. When placed in dilute alkali, carnosol dissolves and the solution undergoes a color change from a beginning orange-red to a deep brown to blue-black. It is also soluble in concentrated sulfuric acid, producing a yellow color. Carnosol is insoluble in water, dilute hydrochloric acid and a 5% solution of sodium bicarbonate.

This paper reports the investigations made concerning the chemical nature of this new compound which is of special interest because of the possibility that it is responsible for the bacteriological activity of extracts from which carnosol is obtained (1). Because of the insolubility in culture media of free carnosol and because of its apparent decomposition in alkali, it has been impossible to determine whether it has bacteriological activity or not.[1] It is hoped that an understanding of the chemical nature of carnosol will make it possible to prepare derivatives suitable for further bacteriological study.

EXPERIMENTAL

Reaction Tests.—A 0.1% solution of carnosol in chloroform slowly and incompletely decolorized a few drops of a 1% solution of bromine in chloroform. However, when a 2% solution of potassium permanganate in acetone was added to a 1% solution of carnosol in acetone, rapid decoloration of the permanganate with production of considerable manganese dioxide occurred. When 0.1 Gm. of carnosol was dissolved in 5 cc. of hot ethanol and 0.1 Gm. of phenylhydrazine added, no cloudiness or precipitate formed upon cooling, dilution or standing. When a drop of ferric chloride test solution was added to a 0.1% solution of carnosol in ethanol, a deep brilliant green color was produced.

* Abstracted from a part of the thesis presented to the Graduate Faculty of the University of Minnesota by Allen I. White in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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