CHEMICAL INVESTIGATIONS ON SKIMMIA LAUREOLA SEEDS

FUAD PASHA*

Chemistry Department, Government College, Lahore

AND

M.K. BHATTY, S.A. KHAN AND KARIMULLAH

West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore

(Received November 17, 1965)

Skimmia laureola (N.O. Rutaceae) seeds have been analysed for their oil and protein constituents. The oil has been shown to contain palmitic (8.28%), stearic (1.47%), palmitoleic (2.57%), oleic (33.40%), linoleic (31.15%) and linolenic acid (23.12%) and the proteins are constituted of aspartic acid, argenine, alanine, glutamic acid, glycine, lucine, isolucine, lycine, prolein, methionine, serine and threonine. The presence of pelargonin and an alkaloid, possibly skimmianine in the seeds, is also indicated.

Skimmia laureola (N.O. Rutaceae), locally known as Nair, is an evergreen shrub that grows throughout the temperate Himalayas at a height of 7,000 to 9,000 feet. In West Pakistan the plant is abundantly available from the Swat State and the Nathia Galli hills. In the indigenous system of medicine, burning of the *Skimmia laureola* leaves near the small pox patients is believed to have curative effects.[1] The plant bears large quantities of small oval-shaped red seeds which have not been put to any practical use so far. That the seeds might become a valuable commodity in the minor forest products, an investigation of their principal constituents was essentially called for. In this paper, the results of this investigation have, therefore, been presented.

The present findings were started with the 'Nair' seeds collected from the Nathia Galli Hills. The red-coloured seed coatings were removed and the seeds were extracted exhaustively with petroleum ether (40-60°). The defatted material thus obtained was preserved for the extraction of the proteins and the alkaloid.

Experimental

The seeds of *Skimmia laureola* (20 g.) were extracted with petroleum ether (40-60°) in a Soxhlet extractor till no oil was being extracted. The extracts were dried over anhydrous sodium sulphate and then filtered. The solvent was removed and the residue was dried at 65-70° for half an hour and then weighed (5.36 g. 26.8%).

The following characteristics of this oil were determined according to the standard procedures.[2]

Refractive index	= 1.4820 at 21°
Acid value	= 5.68
Saponification value	= 100.10
Iodine value	= 154.35
Non-saponifiable matter	= 6.8%

Fatty Acid Composition of 'Nair' Seed Oil.—The oil (3.0 g.) was saponified with 0.5N alcoholic potassium hydroxide (50 ml.) and the fatty acids (1.823 g.) were liberated from the soaps by the standard method.[3] These acids were then converted to their methyl esters by treatment with acidic methanol under reflux for 5 hours. The methyl esters were then analysed by gas-liquid phase chromatography using a 16% DEGS (Diethylene glycol succinate) column at 190 with chart speed of 20″/hr. Nitrogen was used as the carrier gas at a flow rate of 50 ml./minute. In all, six peaks were detected. From the peak areas, the percentage composition of the acids was calculated and is given below:

Palmitic acid (8.28%), stearic acid (1.47%), palmitoleic acid (2.57%), oleic acid (33.41%), linoleic acid (31.15%) and linolenic acid (23.12%).

Amino Acid Composition[4] *of 'Nair' Seed Proteins.*—Total nitrogen was determined according to the procedure of Ashraf, Bhatty and Shah.[5] It was then multiplied by 6.25 (protein factor) to get the total amount of proteins in the seeds (21.3%).

(a) *Acidic hydrolysis*: The defatted 'Nair' seeds (1 g.) were hydrolysed with hydrochloric acid (6N) for 24 hours on a steam bath. The liberated amino acids, present in the filtrate, were dissolved in 10% aqueous isopropyl alcohol (20 ml.) after the filtrate had been heated to dryness.

(b) *Alkaline hydrolysis*: The defatted 'Nair' seeds (1 g.) and a solution of barium hydroxide

*Present address: 10/363, Circular Road, Outside Kashmiri Gate, Lahore-6.

in water (14 %) were heated in an oil bath for 20 hours at 125°. Sulphuric acid (IN) was then added to the cold mixture till the formation of barium sulphate ceased. The amino acids thus liberated were taken up in 10% aqueous isopropyl alcohol (20 ml.) after the filtrate had been heated to dryness as in (a).

Qualitative Paper Chromatography.—The amino acids, as obtained in (a) and (b) above, were separately analysed by paper chromatographic techniques on a Whattman No. 1 filter paper using *n*-butanol: glacial acetic acid: water (4:1:5) in the unidimentional, and *n*-butanol: glacial acetic acid: water (4:1:5) and phenol: water (80:20) solvent systems in the bidimentional procedure. The chromatograms were developed by spraying a solution of ninhydrin (1 % in acetone) and the spots were fixed by further spraying with a solution of saturated aqueous cupric nitrate (1 ml.) in ethanol containing 10% nitric acid. The observed R_f. values along with the possible amino acids were further confirmed by observing identical R_f. values for a synthetic mixture of these amino acids.

Quantitative Paper Chromatography.—The amino acids obtained by the acidic hydrolysis of 'Nair' seed proteins were paper-chromatographed bidimentionally in three sets of experiments with 20, 30 and 40 microlitres of the hydrolysate for each set respectively. The developed spots (with ninhydrin spray) were eluted with a solution of copper sulphate (0.2 mg.) in 20% aqueous ethanol (10 ml.) separately. The resulting solutions were examined colorimetrically at 540 (Beckman DB) after they had stayed at room temperature for 25 hours. The percentage composition of the various amino acids was determined from the comparison of percent transmission with the standard graphs for the amino acids.

Colouring Matter.—Petroleum extraction of the red-coloured 'Nair' seed coatings did not furnish any material. These coatings were then extracted with 1% hydrochloric acid in methanol and the colouring matter was precipitated from the concentrated extracts, by the addition of a large excess of ether. The precipitate was purified by first dissolving it in methanol containing (10% w/v) HCl and picric acid and then reprecipitating it with the addition of ether to the acidic methanol solution (10% hydrochloric acid in methanol) of the picrate. The colouring matter was treated with concentrated hydrochloric acid at 90° (water bath) for five minutes and then extracted with isoamyl alcohol. The anthocyanidin, in the extract, gave various positive colour reactions which identified it as pelargonin.[6] This was further confirmed when the colouring matter was examined by paper chromatography in *n*-butanol: acetic acid: water (4:1:5) solvent system for 10 hours. The spots were developed by spraying a solution of alcoholic aluminium chloride (1%), R_f. value–0.36. The colouring matter was also examined by ultraviolet spectrophotometer when three maxima at M μ 269-270, 331 and 351 were obtained in the 210-600 region, the reported absorption maxima for pelargonin being M μ 269-270, 331 and 351.[6]

Discussion

Two other species of the Rutaceae family, *Skimmia japonica* and *Skimmia repens*, are found in Japan, but not in India and Pakistan. The isolation of an alkaloid, Skimmianine, has been reported from the leaves of both the varieties.[7] The composition of the oil of *Skimmia repens* seeds has also been reported and it was shown to contain oleic, linoleic, linolenic, palmitic and stearic acids.[8]

It has now been found that the seeds of *Skimmia laureola* are rich with regard to both the oil (26.8%) and the protein (21.3%) contents. The oil was analysed for its fatty acid composition of Gas-liquid phase Chromatographic technique, as this procedure is simple and accurate. The identity and percentages of the various acids was determined from the retention times and the peak areas of their methyl esters respectively. The high percentage of unsaturated acids (90.25%) present in the oil suggests that this oil should be an excellent substitute for any other unsaturated oil.

The *Skimmia laureola* seed proteins were hydrolysed under both acidic as well as alkaline conditions separately. The hydrolysates were separately analysed by paper chromatography both unidimentionally and bidimentionally. The bidimentional paper chromatography was found to be more useful not only for qualitative determination of the constituent amino acids, but also for their quantitative determination. The observed R_f. values and the percentage of the amino acids thus determined in the seed protein are given in Table 1.

Preliminary experiments showed that the seeds contain a minor amount of an alkaloid. This alkaloid was examined by thin layer chromatography on a silica gel plate using chloroform: ethanol (9:1) solvent system. The dried plate was sprayed with chloroplatinic acid solution

TABLE 1.—AMINO ACID COMPOSITION OF 'NAIR' SEED PROTEINS ALONGWITH THEIR R_f VALUES AND PERCENTAGES.

Sr. No.	Name	R_f. value	Percentage
1.	Aspartic acid ..	0.08	1.50
2.	Lysine ..	0.26	1.50
3.	Glutamic acid ..	0.27	3.60
4.	Serine* ..	0.29	2.80
5.	Glycine* ..	0.38	2.00
6.	Arganine ..	0.40	2.40
7.	Threonin* ..	0.42	1.00
8.	Methionine ..	0.50	1.30
9.	Alanine ..	0.57	0.50
10.	Lucine ..	0.76	0.12
11.	Isolucine ..	0.83	0.40
12.	Protein ..	0.94	1.10

* Not detected in unidimentional technique.

and revealed only one spot. The observed R_f. value (0.88) suggests that this alkaloid is probably Skimmianine. Since the extracted amount of this alkaloid was very small, it could not be crystallised.

The identification of the anothocyanin was achieved by the examination of the sugar free pigment, with regard to its R_f. value, colour reactions and absorption spectra. Since petroleum ether did not extract any material from the red coloured *Skimmia laureola* seed coatings, the presence of any carotenoids was excluded.

Acknowledgement.—The authors are grateful to Mr. Iftikhar Ahmad, Research Officer of the West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore, for helpful suggestions and discussions.

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