STUDIES ON CITRULLUS COLOCYNTHIS SCHRAD.

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Two crystalline sterols have been isolated from the roots of *Citrullus colocynthis* Schrad., which differ from those isolated so far, and have been tentatively named citrullus colocynthis sterols A and B. Isolation of the alkaloid colocynthis in a crystaline form could not be achieved.

Citrullus colocynthis Schrad., locally known as 'indrayan' or 'tumbi', grows wild in the sandy lands of the north-west, the Punjab, Sind, central and southern parts of Pakistan and India. No where in the subcontinent is it systematically grown. The plant finds mention in the Indian Pharmaceutical Codex 1953 and also in Nadkarni's Indian Materia Medica. According to the latter, all parts of the plants contain traces of an alkaloid and a bitter principle called colocynthin; but since the alkaloid was present only in a very minute quantity, it could not be isolated. It has been mentioned further (under the physiological action of the plant) that in moderate doses it is a drastic hydrogogue, cathartic and diuretic. The physiological properties are attributed to the bitter principle called colocynthin. The different parts of the plant have long been used with advantage in the Indo-Pakistan sub-continent as a drastic purgative in ascites, jaundice and in various uterine conditions.

According to the Indian Pharmaceutical Codex, 1953, page 73, colocynth (dried pulp of the fruit) contains a bitter, amorphous, purgative alkaloid and an amorphous, purgative resin, a small amount of an amorphous glycoside, and the following physiologically inactive substances : α-elaterin, a sterol called citrullol, a hydrocarbon hentriacontane, a phytosterol and a mixture of fatty acids. The seeds contain about 15% of a fixed oil, traces of an alkaloid, an enzyme and a phytosterol.

Even before the start of the present century, sufficient investigation of the plant had already been done by several workers. Among the earliest workers, Walz[1] reported the presence of an amorphous glucoside, colocynthin, $C_{56}H_{84}O_{23}$, and a crystalline substance colocynthitin, the data of which were not given. Walz was followed by other workers like Henke,[2] and Johannson[3] but nothing notable was reported. Maylor and Chappel[4] succeeded in crystallising the colocynthin as pale yellow needles.

Later, Power and Moore[5] claimed that colocynthin and colocynthetin were not definite individuals. They reported the isolation of the following substances from the pulp: (a) A dihydric alcohol citrullol, $C_{22}H_{36}O_2(OH)_2$, m.p. 285 – 290°C. (no rotation is mentioned), this on acetylation furnishing a diacetyl compound, m.p. 167°C.; (b) an amorphous alkaloid; (c) α-elaterin, proposed formulae : $C_{22-28}H_{28-38}O_{6-7}$, m.p. 233°C.; (d) a hydrocarbon, hentriacontane $C_{13}H_{64}$, m.p. 68°C., optically inactive ; (e) a phytosterol, $C_{27}H_{46}O$, m.p. 160 – 162°C.; and (f) some essential oils and fatty acids (few details given).

Agarwal and Dutta[6] in 1934 confirmed the above work and reported the isolation of the same α-elaterin and hentriacontane from the roots of the plant. Next in sequence are Alim Chandani and Tuman Katti[7] whose investigation led to the isolation of a hydrocarbon, m.p. 59–60°C., and three phytosterols, melting at 122–125°C., 288–290°C., 98–100°C., respectively; rotatory power and analysis were not given. B. Hamilton and W. O. Kermack[8] obtained a sterol, α-spinasterol, m.p. 164–165°C., from the fruits.

Comparatively recently, Rafat Siddiqui, Iqbal Rafat Siddiqui and Sultan Mohammad[9] working on the fruits isolated: (a) a semi-crystalline powder citrulluin, $C_{21}H_{30}O_7$, m.p. 125–127°C.; (b) a micro-crystalline powder citrulluene, $C_{21}H_{32}O_{10}$, m.p. 175–178°C.; and (c) a crystalline acid citrulluic acid, formula not given, m.p. 109°C.

In the present investigation work was taken up on the roots of the plant because they had so far attracted relatively lesser attention. The primary object of the work was the isolation of its alkaloid which had evaded all efforts aimed at crystallisation or crystalline salt formation. In spite of all efforts, this could still not be achieved, but during the course of investigation two sterols could be isolated, which differ from those isolated so far, and have tentatively been named as citrullus colocynthis sterols A and B.

First Processing

In an attempt to obtain the alkaloid, the present authors followed two different courses for

processing. In the first, coarsely crushed roots of *Citrullus colocynthis* Schrad., obtained from Hamdard Dawakhana, were directly extracted with alcohol (six times), when, on removal of the solvent, a dark brown viscous residue could be obtained. Except the inorganic salts, which were found to be mainly sodium and potassium chloride, no crystalline substance was visible in the residue. In accordance with earlier reports, the alcoholic residue, when treated with slightly acidified water and tested for alkaloid, gave a strong positive test. The entire alcoholic residue was treated with glacial acetic acid, when a small amount of a white crystalline residue separated out. From this insoluble residue, the two sterols citrullus colocynthis sterols A and B could be isolated, while the glacial acetic acid solution was treated as follows:—

Attempts to Isolate the Alkaloid.—The glacial acetic acid solution was exhaustively extracted with petroleum ether (60 – 80°C.) when a greenish yellow oil was obtained from the petroleum ether extract while a thick dark-brown jelly-like residue separated out. This residue was poured portion-wise with constant stirring into water, when a light-red aqueous solution showing a positive alkaloidal test was formed, while a semi-solid brown residue separated out, which became a solid powder on rubbing with more water. The brown powder could not be crystallised from any solvent, in most of which it was soluble, and did not contain alkaloid any more. The slightly acidic (pH 5) alkaloidal aqueous solution did not give any copious precipitate with concentrated ammonia, 2N sodium carbonate or 2N sodium hydroxide. There appeared a slight turbidity on addition of sodium hydroxide, which was extracted with ether but gave a negative alkaloidal test.

It was finally decided to subject the water-soluble alkaloidal residue to adsorption analysis on alumina according to Brockmann (E. Merck), using methyl alcohol as eluent. None of the nearly 200 fractions collected contained any crystalline material or could be induced to crystallise. It was not possible to separate the alkaloid even in a concentrated form.

The residue of the red solution, however, did not show any visible salt formation with perchloric acid, flavianic acid, reinecke acid, picric acid, oxalic acid and sulphuric acid. Extensive efforts to isolate a crystalline substance from this brown brittle residue by the process of fractional crystallisation, employing the usual organic solvents, also failed.

Treated with 2N sulphuric acid, the residue of the alcoholic extract behaved in the same way as with acetic acid except that a darker red solution was obtained besides the brown paste. On adding concentrated ammonia to the sulphuric acid solution, a precipitate in the form of an emulsified oil appeared at pH 2-3, which went into solution on addition of more ammonia; the solution still remained slightly acidic. The oil went in solution with sulphuric acid as well as with ammonia, thus showing its amphoteric character.

Similarly the 2N sodium carbonate extract of the residue of the alcoholic extract gave a turbidity on acidification with 2N sulphuric acid and also a positive alkaloidal test. 2N Sodium hydroxide, however, dissolved the residue of the alcoholic extract much quicker and without leaving any appreciable residue at all, thus indicating the phenolic nature of the residue. A phenolic resin seems, therefore, to be present besides an amphoteric alkaloid.

Esterification.—Based on the above assumption, it was thought feasible to esterify the alcoholic residue with methanol and hydrochloric acid gas to convert the amphoteric alkaloid into a base. A black reaction product, from which nothing could be isolated, indicated a sensitivity against strong acids. The esterification was therefore repeated with diazomethane and applied on the residue of the aqueous extract after making it free from the above-mentioned phenolic resin. A gas evolution proceeded smoothly, but the dark viscous residue could not be recrystallised from any solvent. Being still soluble in water, no precipitate with alkali could be expected and was also not observed.

The residue was therefore dissolved in 2N acetic acid and precipitated with potassium bismuth iodide reagent. The orange-coloured complex was filtered, dried and decomposed with hydrogen sulphide in moist acetone solution. The clear light-coloured acetone solution was brought to dryness in vacuum. A very hygroscopic residue mixed with inorganic salts was obtained. Half of the amount was passed through a charcoal column (neutral), while the other half was treated with an ion-exchange resin (Biodeminrolet from Permutit, England).

Charcoal Column.—The concentrated aqueous solution was passed through the column, when only inorganic salts were obtained. The alkaloidal part seemed to be adsorbed. After being freed from inorganic salts, alcohol brought out a yellow non-crystalline substance which gave a positive alkaloidal test. This could neither be crystallised nor was there any salt formation possible with picric, flavianic, Reinecke and oxalic acid.

Ion-exchange Resin.—The aqueous solution after treatment with resin was found to be free of inorganic salts and showed a positive alkaloidal test, but here also all attempts to get something definite failed.

The decomposition of the potassium bismuth iodide complex with silver hydroxide furnished an alkaloid-containing mass in good yield, but again this could neither be crystallised nor could it be converted into salts with the help of acids.

Isolation of Citrullus Colocynthis Sterol A.—The white residue obtained by dissolving the alcoholic extract in glacial acetic acid could, on treatment with ethyl acetate, be separated into a soluble and insoluble fraction. From the soluble fraction, citrullus colocynthis sterol A was isolated as nice colourless needles. Three recrystallisations from a mixture of ethyl acetate and methyl alcohol yielded a pure substance melting at 153–154°C., and showing no rotation in 1% chloroform solution; found C, 83.91; H, 11.68; O, 4.72; mol. wt., 343 (Rast camphor). This fits within the range $C_{22-26}H_{36-44}O$; mol. wt., 316.51 – 372.60. The infrared spectrogram (Fig. 1) showed a strong band between 3000 – 3500 cm.$^{-1}$ suggesting a hydroxyl group. Both the digitonin and Lieberman-Buchardt tests were positive, indicating it to be a 3-β-hydroxy steroid, carrying a double bond.

In order to confirm the presence of the hydroxyl group, the sterol A was acetylated. The acetyl compound recrystallised from alcohol as colourless needles, m.p. 134–135°C.

Isolation of Citrullus Colocynthis Sterol B.—The ethyl acetate insoluble fraction was exhaustively washed with water; the water washings were found to contain merely inorganic salts. The residue was recrystallised from alcohol as colourless plates, m.p. 265 –266°C. (with decomposition). It showed a rotation of $[\alpha]\ ^{32}D = -37.89°$ in 1% pyridine solution. The infrared spectrogram showed a strong band between 3000–3500 cm.$^{-1}$. Liebermann-Buchardt test was positive, while the digitonin test was negative, suggesting it not to be 3-β-hydroxy sterol.

Found: C, 72.59; H, 9.99; O, 17.12. This fits within the range $C_{22-23}H_{36-40}O_4$, m.w., 364.1 – 380.55. The presence of four oxygen atoms in the substance would possibly make it a suitable starting material for the synthesis of cortisone. Such an attempt, however, could not be undertaken because of the relatively small yield of the sterol.

On boiling with acetic anhydride and a few drops of pyridine, two acetyl groups entered the molecule, indicating that there are at least two hydroxyl groups susceptible to acetylation under these conditions. The infrared spectrogram in between 1500 to 2000 cm.$^{-1}$ gave no indication of any carbonyl or ester grouping . Also, there was no band round about 1100 cm.$^{-1}$ indicative of a C-O-C band. Therefore nothing can be said about the function of the remaining two oxygen atoms so far. Surprisingly, on de-acetylation the starting sterol was received back with its unchanged rotatory power, $[\alpha]^{28}D = -33.9°$ in 1% pyridine solution, while the acetyl derivative showed no rotation.

It may be mentioned that citrullus colocynthis sterol B has some similarity with citrullol of Power and Moore in so far as this substance also furnishes a formula of $C_{22}H_{38}O_4$; from which only two oxygen atoms seem to belong to hydroxyl group susceptible to acetylation. The m.p.'s however differ and, while Power and Moore could recrystallise their substance from pyridine with a m.p. of 285–290°C. (with decomposition), the

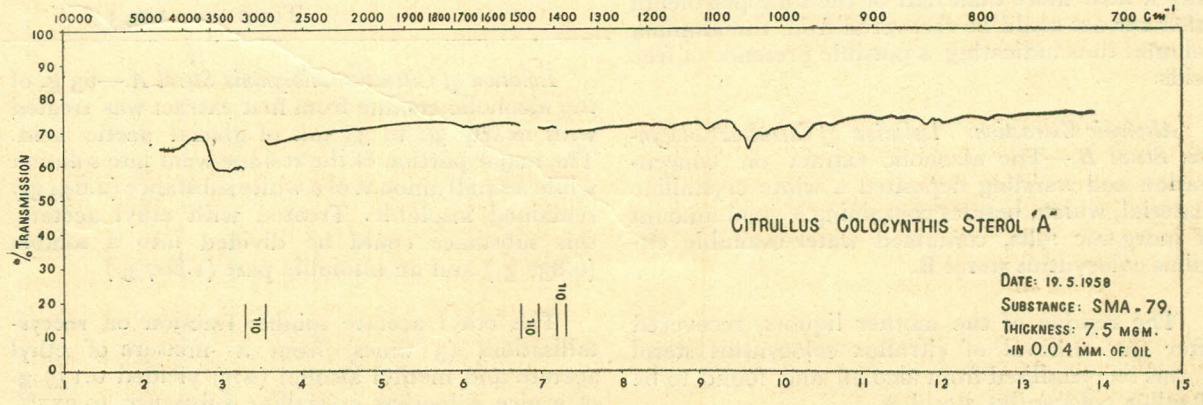

Fig. 1.—The infrared spectrogram of citrullus colocynthis sterol A.

present authors tried in vain to purify their substance from pyridine. After three crystallisations, the m.p. dropped down from 258°C. of the starting raw material to something like 160°C. However, it was found easier to get a pure substance from an alcohol solution. The m.p. of 265 – 266°C. as well as the rotation of $[\alpha]_D^{32} = -37.89°$ remained constant on repeated recrystallisations. The results of analysis were, however, identical with those obtained by Power and Moore.

Second Processing

The coarsely crushed plant roots were first extracted exhaustively with petroleum ether (60 – 80°C.). On removing the solvent, a dark greenish oily mass was obtained, which started depositing a crystalline material on standing. The oily mass was subjected to adsorption analysis on alumina, Brockmann (E. Merck), using petroleum ether, ethyl acetate, alcohol and ether as eluents. Preliminary tests made it possible to combine the 200 fractions collected into seven different groups.

Isolation of Citrullus Colocynthis Sterol A.—The group VI consisted mainly of a crystalline substance. Solubilities and melting point showed it to be identical with citrullus colocynthis sterol A, which had already been isolated during first investigation.

The group No. I, which was mainly an oil mixed with a small amount of a crystalline material, was separated from it by means of adsorption on alumina. The oily parts were distilled under high vacuum. Of the six fractions received, the first two were pure oils while the rest were pasty masses. Fraction V could be sublimed, when four further fractions were obtained. Fraction III was recrystallised from alcohol and found to be citrullus colocynthis sterol A.

It may be mentioned in this connection that only a little more than half of the total petroleum ether extract could be recovered from the alumina column, thus indicating a possible presence of free acids.

Alcoholic Extraction. Isolation of Citrullus Colocynthis Sterol B.—The alcoholic extract on concentration and standing deposited a white crystalline material, which, besides containing a good amount of inorganic salts, contained water-insoluble citrullus colocynthis sterol B.

The residue of the mother liquors, recovered after the removal of citrullus colocynthis sterol B, was recrystallised from alcohol and found to be citrullus colocynthis sterol A.

Experimental

First Method: Extraction with Alcohol

Fresh roots of *Citrullus colocynthis* Schrad. were coarsely powdered in a ball mill as much as possible and extracted with alcohol.

Experiment 1.—1800 g. of the fresh roots were extracted with 34.5 litres of alcohol at room temperature of 32°C., and 119.5 g. of solvent free dark brownish residue (6.6% of the fresh root) was obtained.

Extraction	Time	Solvent	Residue
1	4-7 days	6 litres	67.47 g.
2	,, ,,	6 ,,	26.8 ,,
3	,, ,,	6 ,,	15.4 ,,
4	,, ,,	5½ ,,	2.4 ,,
5	,, ,,	5½ ,,	4.0 ,,
6	,, ,,	5½ ,,	3.5 ,,
		Total =	119.5 ,,

Experiment 2.—Another 1800 g. of fresh roots were extracted with 36 litres of alcohol and 126.3 g. of the solvent free residue (7.01% of the root) was obtained.

Extraction	Time	Solvent	Residue
1	4-7 days	6 litres	73.2 g.
2	,, ,,	6 ,,	31.2 ,,
3	,, ,,	6 ,,	12.0 ,,
4	,, ,,	6 ,,	4.2 ,,
5	,, ,,	6 ,,	3.7 ,,
6	,, ,,	6 ,,	2.0 ,,
		Total =	126.3 ,,

Isolation of Citrullus Colocynthis Sterol A.—63 g. of the alcoholic residue from first extract was treated with nearly 30 to 35 ml. of glacial acetic acid. The major portion of the residue went into solution while a small amount of a white substance (2.644 g.) remained insoluble. Treated with ethyl acetate, this substance could be divided into a soluble (0.837 g.) and an insoluble part (1.807 g.)

The ethyl acetate soluble fraction on recrystallisations (3 times) from a mixture of ethyl acetate and methyl alcohol (2:1) yielded 0.175 g. of a nice colourless crystalline substance (0.277%

of the first alcoholic residue). The m.p. was found to be sharp at 153–154°C. It showed no optical rotation in 1% chloroform solution. Found: C, 83.91; H, 11.68; O, 4.72.3; m.w. (Rast), 342. This fits within the range $C_{22-26}H_{36-44}O$(m.w. 316.51–372.60). Digitonin and Liebermann-Buchardt test were positive. The infrared spectrogram showed the presence of an OH group (shown in Fig.1). It may be named 'citrullus colocynthis sterol A' as long as it cannot be identified with any known sterol.

The substance was readily soluble in chloroform, moderately so in alcohol, acetone, benzene, petroleum ether, ethyl acetate and only sparingly so in methyl alcohol. The quantity of citrullus colocynthis sterol A sharply declined in the subsequent alcoholic extracts.

Acetylation of Citrullus Colocynthis Sterol A.—180 mg. of the sterol A was shaken in a 10 ml. flask with 0.3 ml. of acetic anhydride and a few drops of pyridine for five hours. The crude mass obtained on standing overnight weighed 0.144 g. It was recrystallised from a mixture of ethyl acetate and methyl alcohol as colourless crystals. Yield, 0.0876g.; m.p. 134–135°C. This also showed no rotation in 1% chloroform and was readily soluble in ethyl acetate, chloroform, carbon tetrachloride, moderately so in benzene, acetone, petroleum ether, alcohol and only sparingly in methyl alcohol.

Isolation of Citrullus Colocynthis Sterol B.—1.8 g. of the ethyl acetate insoluble fraction was extracted exhaustively with water until the washings gave no precipitate with silver nitrate. The water washings were brought to dryness and found to contain sodium chloride and potassium chloride. 0.3238 g. of the substance remained insoluble in water. The quantity decreased in later alcoholic extracts.

In order to get more material, the rest of the alcoholic extract was treated likewise when 1.3 g. of the crude ethyl acetate and water insoluble substance was obtained. On recrystallisation from alcohol, wherein it was sparingly soluble, colourless plates weighing 0.548 g. were obtained. After 3 more recrystallisations the m.p. remained constant at 265–266°C. (with dec.) the rotation in 1% pyridine was $[\alpha]^{32}_{D} = -37.89°$. Found: C, 72.59; H, 9.99; O, 17.12. This fits within the range $C_{22-23}H_{36-40}O_4$, m.w., 364.1–380.55. The infrared spectrogram indicated the presence of OH groups (shown in Fig. 2). This sterol may be called 'citrullus colocynthis sterol B.' It gives a positive Liebermann-Buchardt test but a negative digitonin test.

It was readily soluble in pyridine, tetrahydrofuran, dimethylformamide, moderately so in benzene, carbon tetrachloride and only sparingly in alcohol and acetone. It was insoluble in water, ethyl acetate and petroleum ether.

Acetyl Compound of Citrullus Colocynthis Sterol B.—0.373 g. of sterol B together with 4 ml. of acetic anhydride and a few drops of pyridine were refluxed for five hours in a 25 ml. flask. On leaving overnight the acetyl compound crystallised out. It was filtered on mild suction. Yield, 0.1543 g. crude. On several recrystallisations from alcohol, 0.1543 g. of colourless finely crystalline substance was obtained. The m.p. was constant at 159 – 160°C. It showed no rotation in 1% chloroform solution.

It was easily soluble in acetone, benzene, chloroform, ethyl acetate, dimethylformamide, moderately so in alcohol and sparingly in petroleum ether. The report of analysis showed that the acetyl sterol B contained 20.7 % of ($CO.CH_3$) group which would agree with the presence of

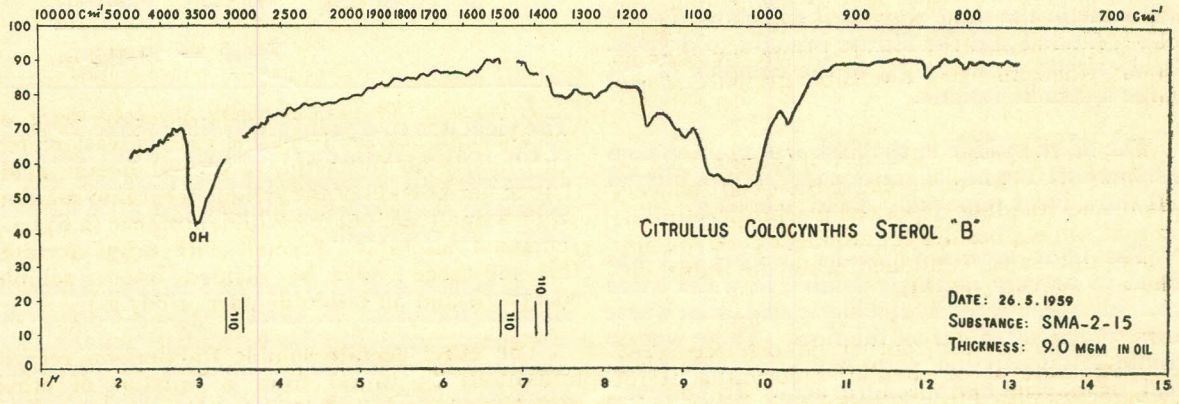

Fig. 2.—The infrared spectrogram of citrullus colocynthis sterol B.

2 hydroxyl groups in its molecule susceptible to acetylation under these conditions. Calculated: $C_{22}H_{34}O_2(O\text{-}COCH_3)_2$, (19.1%),

Deacetylation of Colocynthis Sterol B.—100 mg. of acetyl sterol B was refluxed on a water bath for 5 hours with 60 mg. of potassium hydroxide in alcohol solution and left overnight. The alcohol was removed and the residue treated with water, filtered from the insoluble which was again washed with water until it gave no alkaline test with litmus. The substance was recrystallised from alcohol. Yield, 0.0195 g. The deacetylated compound showed a rotation of $[\alpha]_D^{28} = -33.9°$ in 1% pyridine, which agrees with that of the original sterol B. It melts at 265 – 266°C. Solubilities were similar to the original sterol B.

Precipitation of an Alkaloid.—47.5 g. of the alcoholic residue was shaken three times for a total period of an hour with 50 ml. of water each, slightly acidified with acetic acid. The solution was always decanted off from a brown viscous water-insoluble resin. The third extract gave only a weak test with potassium bismuth iodide reagent. The residual oily resin weighed 20.73 g. after drying over phosphorous pentoxide at room temperature.

The three combined extracts were precipitated with potassium bismuth iodide reagent and the resulting orange coloured substance filtered by suction, washed with water, and dried on a porous plate. Yield, 18.2048 g. or 38% of the alcoholic residue.

Potassium bismuth iodide complex (1.6 g.) was shaken for 5 hours in a 250 ml. glass stoppered flask containing a few glass beads and 3 g. of freshly prepared silver hydroxide and 100 ml. of acetone. It was filtered and the filtrate treated a second time with a fresh quantity of silver hydroxide in the same way, as it still showed some colour, filtered and the filtrate brought to dryness. Yield, 0.2303 g., i.e., 14% of the complex of 5% of the alcoholic residue.

The black residue in the flask was again shaken with another 100 ml. of acetone for 5 hours, filtered and the solvent removed. Yield, 0.0082 g.

The light brownish powder (0.2303 g.) was found to be very sparingly soluble in water more easily in concentrated hydrochloric acid, from where it was precipitated out on dilution. There was no visible salt formation with flavianic acid, picric acid, oxalic acid and sulphuric acid. It, however, gave a thick precipitate with usual alkaloidal reagents in acidic solutions.

Second Method: Extraction with Petroleum Ether

Experiment 1.—2 kg. of coarsely powdered roots of *Citrullus colocynthis* Schrad. were extracted six times with petroleum ether (b.p. 60 – 80°C.) at room temperature (32°C.) as follows:

Extraction	Time	Solvent	Residue
1	24 hours	8 litres	5.6017 g.
2	48 ,,	8 ,,	2.5410 ,,
3	48 ,,	7½ ,,	1.0890 ,,
4	48 ,,	7½ ,,	0.6045 ,,
5	48 ,,	7½ ,,	0.6297 ,,
6	48 ,,	7½ ,,	0.3713 ,,
		Total =	10.8372 ,,

Solvent-free green oily residue, 10.8372 g. (0.541% of the root) was obtained, which on standing for several days deposited increasing amount of a crystalline material.

Experiment 2.—Another 2 kg. of roots were extracted with a total of 49 litres of petroleum ether (b. p. 60 – 80°C) by percolation at room temperature (32°C), according to the following scheme.

Extraction	Time	Solvent	Residue
1	48 hours	8½ litres	5.8360 g.
2	48 ,,	8 ,,	2.2265 ,,
3	48 ,,	8 ,,	1.2060 ,,
4	48 ,,	8 ,,	0.5746 ,,
5	48 ,,	8 ,,	0.3250 ,,
6	48 ,,	8 ,,	0.2670 ,,
		Total =	10.4351 ,,

The yield was 10.43 g. of green oily residue (0.521% of the root). A further quantity of the roots was extracted with petroleum ether in the same way as described above when a total of 53.2 g. of the substance was collected.

Adsorption Analysis of the Petroleum Ether Extract on Alumina (Brockmann, E. Merck).—53.2 g. of the green oily mass containing the crystalline material was dissolved in a minimum quantity of petroleum ether (b.p. 60 – 80°C.), placed on top of a column of 50 cm. length, 3½ cm. width, filled with 279 g. of alumina made wet with 300 ml. of petroleum

ether. Elution was done successively with petroleum ether, ethyl acetate, benzene, alcohol and ether.

A total of 216 fractions were collected taking 50 ml. and later 100 ml. each time. Based on the physical appearance, solubilities and microscopic examination, the collected fractions could be combined into seven different groups as shown in the following table:

Fraction No.	Eluent	Eluent used ml.	Amount in g.	Group No.	Remarks
1- 10	Petroleum ether	50	18.4067	I	Light green oil with small amount of a crystalline material
11- 20	,,	50	2.2026	II	Colourless needles mixed with pasty mass
21- 26	,,	50	0.4170	III	Broad rod shaped crystals with a pasty mass
27- 35	,,	100	0.4744	IV	Substance containing few rods but mostly clusters
36- 62	,,	100	0.6771	V	Mainly cluster-shaped substance with pasty mass
63-150	,,	100	3.2810	VI	Beautiful needles of a colourless crystalline substance
151-190	Ethyl acetate	100	41,160	VII	Light yellow uniform oil
691-207	Alcohol	100	1.8439		
208-216	Ether		0.5398		
		Total ..	31.8458		
		Balance ..	21.4 g. remained in column.		

Investigation of Group VI. Isolation of Citrullus colocynthis Sterol A.—3.28 g. of the crude mass obtained from group VI (fractions 63 – 150) was recrystallised from alcohol. M.p. 153 – 154°C, yield 0.1315 g. It was found to be identical with citrullus colocynthis sterol A (described under first method).

Investigation of Group I.—17.59 g. of the oil mixed with a small amount of crystalline substance from group I (fractions 1–10) was dissolved in a minimum quantity of petroleum ether and passed through a column of alumina according to Brockmann (E. Merck) of 51 cm. length and 2 cm. width containing 120 g. of alumina. Elution was done with petroleum ether and later with ethyl acetate. Fractions of 50 ml. each were collected and the solvent removed. A total of 14.527 g. of a light yellow oil could be recovered in this way which appeared to be uniform in appearance, 3.0 g. of oil remaining in the column. Benzene and chloroform failed to bring down any substance. Twenty oil fractions collected in the way described were mixed together and distilled in high vacuum from a copper block.[10]

The following fractions were obtained.

Fraction	Amount	Temperature	Pressure	Remarks
1	2.0472 g.	130-160°C.	0.06 mm.	Light-coloured oil
2	2.1440 g.	106-190°C.	0.06 mm.	Light-coloured oil
3	0.8945 g.	190-230°C.	0.06 mm.	Mostly oil with a solid substance
4	0.1550 g.	230°C.	0.06 mm.	Solid substance with a little oil
5	0.7660 g.	230-160°C.	0.06 mm.	Solid material with little oil
6	1.4680 g.	260-290°C.	0.06 mm.	Solid mixed with oil (could not be crystallised or sublimed)
Total =	7.4747 g.			

The light-coloured oil from fractions 1 and 2 (339 mg.) was saponified, when a coloured oily alcohol (0.198 g.) and a fatty acid 0.230 g. were obtained. The fatty acid was found to give a urea inclusion compound. It was dried thoroughly over phosphorus pentoxide and then treated with 0.15 ml. of phenyl isocyanate for 5 hours, at 180–185°C. The excess of isocyanate was removed in vacuum. Residue, 0.370 g. (oily mass). In spite of all efforts, the anilide could not be obtained in a pure state due to the small amount. 0.766 g. of the pasty mass from fraction 5 could not be recrystallised from any solvent and so was sublimed under high vacuum.

Four fractions were collected as shown:

Fraction	Amount	Temperature	Pressure	Remarks
1	0.2081 g.	upto 150°C.	0.06 mm.	Pasty mass
2	0.0594 g.	upto 180°C.	0.06 mm.	Pasty mass
3	0.3219 g.	upto 230°C.	0.06 mm.	White solid substance
4	0.0124 g.	upto 270°C.	0.06mm.	Pasty mass
Total =	0.6018 g.			

Fraction No. 3, 0.321 g. of the white substance, was recrystallised from alcohol. Yield 0.0573 g., m.p. 153 – 154°C. The solubilities, crystal shape and m.p. were identical with citrullus colocynthis sterol A (described under first method). All attempts to get crystalline material from the other fractions failed.

Investigation of groups II to VII did not lead to any pure substance.

Extraction with Alcohol.—2 kg. of roots after exhaustive extraction with petroleum ether were extracted with a total of 53 litres of alcohol at room temperature (31°C):

Extraction	Time	Solvent	Amount
1	48 hours	8 litres	33.3 g.
2	48 ,,	8 ,,	28.8 ,,
3	48 ,,	8 ,,	26.0 ,,
4	48 ,,	7½ ,,	20.5 ,,
5	48 ,,	7½ ,,	12.3 ,,
6	48 ,,	7½ ,,	9.1 ,,
7	48 ,,	7 ,,	3.0 ,,
		Total =	132.0 ,,

i.e., 6.6% of the root (dark brown pasty mass)

Isolation of Citrullus Colocynthis Sterol B.—The first alcoholic extract on concentration and standing deposited 1.0921 g. of a white crystalline material. It was filtered and repeatedly washed with water in order to remove the inorganic salts specially sodium and potassium chloride until the washings did not give any precipitate with silver nitrate.

The white residue was recrystallised (three times) from alcohol in which it was sparingly soluble. Yield 0.2765 g., m.p. 265–266°C. On the basis of m.p. and solubilities it was identified as citrullus colocynthis sterol B (described under first method).

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References

1. Walz, N. Jahrb. Pharm, **16**, 225 (1858); (1851), 16, & 10.
2. Henke, Arch. Pharm, 221, 200, (1883).
3. Johannson, Z. Chem, **23**, 154, (1885).
4. Naylor and Chappel, Pharm. J., **79**, 99 (1907).
5. Power and Moore, J. Chem. Soc., 97, 99 (1910).
6. R. R. Agrawal and S. Dutta, Current Science, **3**, 250 (1934).
7. Alim Chandani and Tuman Katti, J. Indian Chem. Soc., **26**, 515 (1949).
8. B. Hamilton and W.O. Kermock, J. Chem. Soc., 5051-2 (1952).
9. Rafat Siddiqui, Iqbal Rafat Siddiqui and Sultan Mohammad, J. Ind. Chem. Soc., 669 (1955).
10. Nisar Ahmad and Georg Hahn, Pakistan J. Sc. Ind. Research, **2**, 53 (1959).