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THE CHEMICAL CONSTITUENTS OF THE LEAVES OF *ACHRAS SAPOTA*

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The amino acids and carbohydrates present in the leaves of *Achras sapota* have been studied. The amino acids present are aspartic acid, alanine, aminobutyric acid, arginine, cystine, glutamic acid, glycine, hydroxyproline, lysine, phenylalanine, proline, serine, tyrosine and valine. Five sugars namely: arabinose, fructose, glucose, galactose and lactose have been identified to be present. The n-hexane extract of the leaves has been found to contain tritriacontane and β-sitosterol as major constituents.

INTRODUCTION

We have previously reported the amino acids, mono-, di-, and poly- saccharides contents of *Achras sapota* L. fruits[1]. Very little studies appears to hvae been undertaken regarding the chemical constituents of *Achras sapota* leaves. Presence of an alkaloid – sapotine has been reported in kernels[2] and bark[3]. Myrecitin and myrecitin-3-0-L-rhamnoside have been found to be present in the leaves[4]. In this communication we are reporting(a) the identification of amino acids and sugars present in the alcoholic extract of the leaves of *Achras sapota* L and(b) the n-hexane soluble constituents of *Achras sapota* leaves.

EXPERIMENTAL

Fresh leaves of *Achras sapota* (1 Kg) were dried at room temperature in the shade and extracted by soaking in 95% ethyl alcohol (3 x 3 litres) for five days each time. The combined ehtanol extract was concentrated under vaccuo at 50° and the following studies were carried out.

Separation of Amino Acids and Sugars in the Extract by Ion-exchange Column Chromatography

A sample of concentrated alcoholic extract (2.5g) obtained after complete evaporation of the solvent was studied for the contents of carbohydrates and amino acids. Ion-exchange column of Dowex (50x4); 0.9 by 30 cms at 40°C was employed for separation. The sugars were eluted with deionised water and the elutes were tested by Molisch test till complete removal of the sugars. The sugars were found to be concentrarted in the earlier fractions (determined by optical density measured at 365 nm). All the sugars containing fractions were combined and concentrated under vaccuo at 40°C and taken in 6 ml. of water. The concentrated mass labelled as FR-1 was kept for further studies. The amino acids were eluted with 5 and 10% ammonia. Out of 60 fractions 40 fractions gave positive test with ninhydrin after removal of ammonia (the elution pattern was determined by measuring the absorbance of each fraction at 375 nm). All those fractions which gave positive test for amino acids were mixed together and concentrated under vaccuo at 40-45°C. The concentrated volume was made up to 5.5ml and labelled as FR-2.

Qualitative Analysis of Sugars. Paper partition chromatography using descending technique was employed for the separation and identification of carbohydrates present in FR-1. The following solvent systems were used: (A) n-butanol:acetic acid: water (4:1:5 –upper layer); (B) n-butanol:ethanol:water (4:1:5 – upper layer); (C) isopropanol: Butanol:water (7:1:2); (D) ethyl acetate:pyridine:water (10:4:3); System D was found to give best resolution and five sugars namely: arabinose (traces), glucose, galactose, fructose and lactose were identified.

Qualitative Analysis of Amino Acids. Qualitative analysis of free amino acids present in the FR-2 was resolved by means of paper chromatography using different solvent systems. Reference amino acids (E. Merck's collection A, B & C) were also spotted on the paper as markers. Since the chromatogram showed a number of unseparated spots, a known quantity of the concentrate FR-2 was hydrolysed

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by 6M-HCl at 120° for 48 hours in vaccum sealed tube. The hydrolysate was filtered and the acid removed vaccuo. The hydrolysate was subjected to the column of freshly regenerated and equilibrated Dowex – 50 x 4 (200-400 mesh). The amino acid fractions were eluted with pyridine-acetate buffer (0.2 M pyridine) at pH 5.5-5.6. About 42 fractions of 2 ml. each were eluted and an aliquot of each fraction was tested with ninhydrin. Most of the amino acids were found in the fractions 5 to 25. The mixed fractions after concentration were spotted on the paper and the paper was run according to a modified system developed by Zaidi and co-workers[5]. The following amino acids were identified: Aspartic acid, alanine, arginine, glutamic acid, glycine, lysine, phenylalanine, proline, serine, threonine and tyrosine.

Quantitative Analysis of the Amino Acids in the Hydrolysate. The quantitative analysis of amino acids present in the hydrolysate fraction was determined using Beckman 120° Automatic Amino acid Analyser. The total amino acids presents alongwith their quantities are shown in Table 1.

n-*Hexane Soluble Constituents of* A. sapota *Leaves.* The resudial material left after extraction from ethanol was further extracted by hot and cold n-hexane. The powdered leaves were shaken in 2.5 litres of *n*-hezane at room temperature on electric shaker for five days in case of cold extraction while the hot extract was obtained using soxhlet apparatus for 72 hours. TLC examination of both cold and hot extracts revealed them to be identical. The extracts were, therefore, combined and concentrated under reduced pressure at 40-45°. Since the TLC examination of crude-n-hexane extract in various solvent systems showed a number of compounds alongwith substantial amount of chlorophyll and other coloured impurities; it was loaded on a short aluminium oxide column and eluted with benzene, whereupon the chlorophyll and coloured material remained behind. The elutes on concentration yielded a yellow gum showing four distinct spots on TLC (silica gel - 0.25 mm thick). The yellow gum was then rechromatographed using a column of neutral aluminium oxide and gradient solvent system. The solvents used were neat hexane followed by 5, 10, 15, 20% benzene in n-hexane and neat benzene. Fractions of 10 ml each were collected and the composition of each fraction was monitored (TLC).

Isolation of Hydrocarbons from Fractions 1-20. Fraction's 1 to 3 did not show any spots on TLC; fractions 4 -13, however, gave a sharp single spots having the same R_f value and were, therefore, combined and concentrated to dryness under vaccuo whereupon a white solid compound, 70 mg; m.p. 69 to 70°C was obtained. Recrystallisation from chloroform:methanol, 1:1 afforded pure cyrstalline substance m.p. 70°. Carbon and hydrogen analysis gave C = 85.4 and H = 14.63%; while mass spectrum showed a molecular ion peak at *m/e* = 464 and a base peak at *m/e* = 57. Other prominent peaks were at *m/e* = 71, 85, 99, 113, 127, 141, 155, 169, 183,. . . with decreasing intensity. The infra-red was that of a typical hydrocarbon, while the NMR spectrum showed a sharp intense singlet at $\delta = 1.27$ and a small distorted triplet at $\delta = 0.87$, typical of a long straight chain hydrocarbon[6]. One the basis of the above, the compound was identified as a straight chain hydrocarbon, tritriacontane, $C_{33}H_{68}$ ($C_{33}H_{68}$ requires C = 85.57 found 85.40 and H= 14.65 found 14.63%).

Table 1. Qualitative analysis of sugars present in the leaves of *Achras sapota* L.by paper chromatography

Solvent system	Elution time	Constituent sugars identified	R_f Sample	R_f Standard
1. BuOH: AcOH Wa (4:1:5)	20 hrs	Fructose	1.09	1.12
		Glucose	1.00	1.00
2. BuOH:EtOH: Wa (4:1:5) upper layer	48 hrs	fructose	1.12	1.13
		Glucose	0.99	1.00
		Galactose	0.90	0.92
		Lactose	0.59	0.58
3. isoPrOH:BuOH:Wa (7:1:2)	20 hrs	Fructose	1.12	1.12
		Glucose	1.02	1.00
		Galactose	0.88	0.90
		Lactose	0.41	0.41
4. EtOAc:Py:Wa (10:4:3)	11 hrs	Fructose	1.09	1.11
		Glucose	0.98	0.99
		Galactose	0.84	0.86
		Lactose	0.47	0.48
		Arabinose	trace	

BuOH= n-Butanol, AcOH = Acetic Acid; Wa = Water; EtOH = Ethyl alcohol; EtOAc = Ethyl Acetate; Py = Pyridine.

Fraction 14-20. also showed single spots having a slightly lower R_f value then that of the compound obtained above. The fraction were combined and concentrated under reduced pressure. On leaving overnight at 0°C, a compound m.p. 40-45° was obtained. Due to small quantity of the compound complete spectral analysis could not be obtained Its infra-red spectra, however, indicated it to be also a

hydrocarbon.

Isolation of β-sistosterol. Fractions 21-30 and 31-45 eluted with 5 and 10% benzene in hexane did not yield any compounds. Fractions 46-55, eluted with 15% benzene in hexane afforded a yellow gum (125 mg) which on TLC examination showed it to consist mainly of one compound with trace of another. The gum was, therefore, takenup in chloroform-methanol (1:1) and allowed to stand overnight at 0°C. A white crystalline substance, m.p. 136-140° was obtained, which on further recrystallisation from the same solvent yielded a crystalline powder, m.p. 139-141°C. The compound was identified as β-sitosterol on the basis of melting point, comparative TLC. its NMR and infra-red spectrum were also identical to that of authentic sample.

DISCUSSION

Previously Sidappa and Bhatia[7] have reported the presence of fructose, glucose and sucrose in the fruits. Presence of lactose has also been reported[8]. In our earlier communication[1],we have confirmed the presence of five sugars in the fruits, namely: fructose, glucose, sucrose and lactose alongwith galacturonic acid. No work on carbohydrates in leaves of *Achras sapota* appears to have been undertaken. In the present studies on the leaves of *Achras sapota* we have found the presence of lactose alongwith fructose, glucose and galactose when examined by paper chromatography. Trace amount of arabinose was also evident in the chromatogram; using the ethyl acetate: pyridine:water (10:4:3) system. Further the quantity of mono- and disaccharides in the leaves of *Achras sapota* appears to be substantially less as compared to that of mono- and disaccharides in the fruits as reported by us earlier. This is not surprising because the polysacchrides in the fruits, breakdown to simpler sugars due to enhanced enzymatic activity in riping fruits; where as the enzymatic activity is not so pronouned in the leaves and most of the carbohydrates remain in the polysaccharide form.

No report with specific reference to amino acids contents in leaves of *Achras sapota* appears in literature. In the present investigation using paper chromatography and automatic amino acid analyser,we have found the presence of the following: alanine, β-alanine, β aminobutyric acid, arginine, aspartic acid, cystine, glutamic acid, glycine, hydroxyproline, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine. A comparative study of the amino acids in our present studies on leaves with that of our previous fiindings on fruits is shown in Table 2.

It seems that the concentration of proline is very prounced in the fruits but low in the leaves. Isoleucine, leucine and methionine are found to be absent in the leaves, while hydroxyproline, β-aminobutyric acid and β-alanine which occurs in susbtantial amount in the leaves are found to be totally absent in fruits. The other amino acids which are found to be present in both fruits and leaves, it has been observed that the contents are more pronounced in leaves as compared to fruits.

In the present studies we have also isolated and indentified a few compounds from the n-hexane extracts of leaves. Amongst them a straight-chain, hydrocarbon, tritriacontane, molecular formula $C_{33}H_{68}$; m.p. 69-70° (lit 71°) was obtained. Its structure was established on the basis of C and H analysis and spectral studies. Another compound m.p. 40-45° was also obtained. It had a very close R_f value to the tritriacontane mentioned above and was also, probably a hydrocarbon since its infra-red spectra showed absence of any other functional group. Complete analysis of this compound could not, however, be obtained due to

Table 2. A comparative study of the amino acids present in the fruits and leaves of *Achras sapota* L.

S. No.	Amino acids identifed	Quantity determined in mole/100 g	
		Fruits	Leaves
1.	Alanine	2.83	–
2.	β-alanine	absent	15.27
3.	β-Aminobutyric acid	absent	15.1
4.	Arginine	16.89	–
5.	Aspartic acid	4.56	14.45
6.	Cystine	Absent	trace
7.	Glutamic acid	3.71	14.54
8.	Glycine	13.79	48.18
9.	Hydrozyproline	–	–
10.	Isoleucine	2.22	absent
11.	Leucine	2.46	absent
12.	Lysine	10.55	–
13.	Phenylalanine	trace	16.0
14.	Proline	41.16	4.0
15.	Serine	10.42	30.0
16.	Threonine	11.63	10.0
17.	Tyrosine	0.75	11.0
18.	Valine	3.50	trace
19.	Methionine	0.83	absent
20.	Urea	–	absent

– = present but values could not be determined due to non-availability of the standard at the time the sample were run.

insufficient quantity. A third compound, m.p. 139-141°, was also isolated from the n-hexane extract of leaves and separated by column chromatography. It was identified as β-sitosterol on the basis of its m.p., comparative TLC and infra-red spectra. While this paper was in press we have been successful in isolating three triterpenoits from /pet. either extracts of the leafs we will be reporting about structures of these compounds shortly.

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