

# BRAZILIAN JALAP

## PART II

### FURTHER HISTOLOGICAL AND PHARMACOGNOSTICAL EXAMINATION OF THE FOUR SAMPLES OF BRAZILIAN JALAP AND SOME COMPARISONS WITH VERA CRUZ JALAP AND ORIZABA JALAP

BY E. J. SHELLARD

*From the School of Pharmacy, College of Technology, Bristol*

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#### INTRODUCTION

IN Part I<sup>1</sup> the anatomical and histological characters of 4 commercial samples of Brazilian jalap were described. It was observed that although all had the same general anatomical structure there were some marked differences histologically. Although these were insufficient to decide whether or not the roots were obtained from the same or different botanical sources, they were sufficient, particularly in the case of Sample A, to prevent Brazilian jalap from being defined histologically with any exactitude. Further investigations of the histological and pharmacognostical characters of these 4 samples have now been carried out with the object of (1) differentiating still further, if possible, between them and (2) establishing some common features by which Brazilian jalap could be definitely identified in the powdered condition and also distinguished from powdered Vera Cruz jalap, *Ipomæa purga* Hayne, and powdered Orizaba jalap, *Ipomæa orizabensis* (Pelletan) Ledanois, particularly when in admixture with these drugs. For this reason some of the characters of Brazilian jalap are compared with those of Vera Cruz jalap and Orizaba jalap.

The histological characters of Vera Cruz jalap and of Orizaba jalap are well defined and are referred to in various text books of pharmacognosy. In those cases where no suitable information was available, examination was made of representative samples of these drugs taken from the Pharmacognosy Museum, College of Technology, Bristol.

#### HISTOLOGICAL CHARACTERS

The main histological features which are useful diagnostically are:—starch, calcium oxalate, cork, vessels and other xylem elements, fibres, stone cells and latex cells.

*Starch.* O. A. Farwell<sup>2</sup> reports the presence of starch "only in a very small amount" in Brazilian jalap, the grains varying from 2 to 24  $\mu$  in diameter, but gives no indication of the shape or appearance. The British Pharmaceutical Codex 1949 describes the starch as being rounded or muller shaped, occurring as simple grains or in compound grains of 2 to 7 components, the sizes of the single grains being 3 to 8 to 16 to 30  $\mu$ . No other description appears to have been recorded other than that in

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Part I<sup>1</sup>. As stated, all 4 samples contain abundant starch varying from 3 to 30  $\mu$ , the majority being from 12 to 18  $\mu$  in diameter. The smaller grains are rounded often in aggregations containing up to 12 grains, while the larger grains are muller shaped and usually single or compound grains of 2 or 3 components. There is no difference between the starches of the 4 samples examined.

The sizes of the starch grains of Vera Cruz jalap are given by various sources as being from 3 to 40  $\mu$  in diameter with some gelatinised grains being up to 65  $\mu$  in diameter. The majority of the grains measure from 24 to 30  $\mu$ . Those of Orizaba jalap are given as being from 2 to 40  $\mu$ , the majority being from 18 to 24  $\mu$ . The shapes of the grains are similar to those of Brazilian jalap. Theoretically it should be possible to differentiate between these varieties of jalap by selecting two suitable starch index numbers, e.g., 18  $\mu$  and 24  $\mu$  and determining the percentage of starch grains in each jalap having diameters greater than these figures, but the abundance of starch present in the drugs would make the measurement of representative selections of grains impracticable.

*Calcium Oxalate.* While O. A. Farwell<sup>2</sup> refers to rosette crystals of calcium oxalate in Brazilian jalap, he makes no reference to their sizes. The only reference available is that in the British Pharmaceutical Codex 1949 which gives the sizes as 15 to 20 to 30 to 40  $\mu$  wide. In Part I of this paper the measurements given are from 8 to 40  $\mu$  and reference is made to the difference in size between those in the phelloderm and those occurring in longitudinal files in the pericyclic parenchyma. In order to determine the limits of the majority of the crystals in each sample of Brazilian jalap, an analysis was made of the length of the longest axis of about 250 crystals from each of the samples.

A small quantity of powdered drug, all of which passed through a No. 85 sieve was mounted in glycerin after clearing by warming with chloral hydrate. By means of a moving stage it was possible to examine the entire mount, strip by strip. Every crystal in the field was measured by means of a calibrated eyepiece micrometer. The results are shown in Table I.

TABLE I  
CALCIUM OXALATE CRYSTALS

Sample	Up to 12 $\mu$	12 to 18 $\mu$	18 to 24 $\mu$	24 to 30 $\mu$	30 to 35 $\mu$	35 to 40 $\mu$	Total Measured
A ... ..	54	84	93	25	17	4	276
B ... ..	28	72	106	36	18	4	264
C ... ..	46	90	85	24	13	3	261
D ... ..	41	108	78	27	7	5	266

It is seen that in each case about two-thirds of the total number of crystals are between 12 and 24  $\mu$  wide. The figures given above do not suggest any difference between the 4 specimens of Brazilian jalap.

The sizes quoted by various sources for cluster crystals in Vera Cruz jalap and Orizaba jalap are 15 to 25 and occasionally up to 30  $\mu$  wide for

the former, and from 15 to 45  $\mu$  wide for the latter. While no analysis of the sizes of the crystals of these drugs was made, they were examined for crystals over a certain size, viz. 30  $\mu$  in the case of Vera Cruz jalap and 45  $\mu$  in the case of Orizaba jalap.

The specimens of Brazilian jalap had been examined for crystals greater than 40  $\mu$  wide during the analysis of their crystal sizes.

In no case was the upper limit exceeded and it can therefore be concluded that the maximum dimensions for the longest axis of the crystals of calcium oxalate are as follows:—Vera Cruz jalap, 30  $\mu$ ; Brazilian jalap, 40 $\mu$ ; Orizaba jalap, 45 $\mu$ .

Prismatic crystals are present in small numbers in Orizaba jalap but not in Vera Cruz jalap. None could be observed in Brazilian jalap.

*Cork.* No references are available for the sizes of the cork cells in Brazilian jalap or Orizaba jalap, but Moll and Janssonius<sup>3</sup> give the sizes of the cork cells in Vera Cruz jalap as 40 to 60  $\mu$  long and up to 30  $\mu$  wide. No figures are recorded for the number of cork cells per sq. mm. of surface area for any of the varieties and although the shape and size of the cork cells vary considerably, it was thought desirable to obtain these figures for reference purposes.

Measurements obtained for the cork cells in surface view are as follows:—Brazilian jalap, 40 to 120  $\mu$  long, 80 to 90  $\mu$  wide; Vera Cruz jalap, 40 to 100  $\mu$  long, 20 to 50  $\mu$  wide; Orizaba jalap, 30 to 85  $\mu$  long, 20 to 45  $\mu$  wide.

The determination of the number of cork cells per sq. mm. of surface area was carried out as follows.

Surface sections of the cork from several specimens of each drug were prepared and bleached by warming with ammoniacal solution of hydrogen peroxide. When the pieces of cork had been bleached sufficiently the solution was made acid with dilute hydrochloric acid; the pieces were removed, thoroughly washed in water and rinsed in ethanol. They were kept in 90 per cent. ethanol until required for examination. By means of a camera lucida and using a 4 mm. objective and a X12 eyepiece an area was drawn on a sheet of paper corresponding to 5 squares of a calibrated netruled eyepiece micrometer. The area of the 5 squares was calculated to be 0.1066 sq. mm. Sections of the cork were then mounted in dilute glycerin and examined. A cross was marked on the paper to correspond with every cork cell within the area. Those cork cells which overlapped the edge of the drawn area were considered to be inside it if more than half the cell was judged to be within the area. The number of crosses was then counted and the number of cork cells per sq. mm. of surface area was calculated. In all cases 20 surface sections were examined and the number of separate counts per section varied from 5 to 10. A summary of the results is given in Table II.

The results obtained suggest that the four samples of Brazilian jalap are obtained from identical species (giving figures for the drug, 307.7 to 425.9 cork cells per sq. mm. of surface area) but that determination of the number of cork cells per sq. mm. would not clearly distinguish between the 3 varieties of jalap under consideration.

*Vessels and other Xylem Elements.* No references are available regarding the dimensions of vessels and other xylem elements of Brazilian jalap other than those given by Farwell<sup>2</sup>, who states that the reticulate

TABLE II  
CORK CELLS

	Counts made	Cork cells per count		Average counts per section		Cork cells per sq. mm.	
		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Brazilian jalap A ...	144	30	48	33·6	43·2	315·2	405·2
„ B ...	138	32	49	34·9	45·4	327·3	425·9
„ C ...	140	30	47	33·4	43·1	313·3	404·3
„ D ...	146	30	48	32·8	44·2	307·7	414·6
Vera Cruz jalap ...	143	26	40	31·2	35·8	292·3	335·0
Orizaba jalap ...	136	36	54	40·2	48·0	377·1	451·5

vessels are often 0·13 mm. in diameter with walls 0·008 mm. thick and that the tracheids average about 0·44 mm. in length, and 0·026 mm. in width and have a wall about 0·003 mm. in thickness. He further states, "The wood fibres have oblique pores and range from 0·62 to 0·78 mm. in length, 0·013 to 0·020 mm. wide and have a lumen varying from 1/5 to 3/4 the width of the fibre." In Part I<sup>1</sup>, dimensions for the diameter of the vessels in Brazilian jalap were given as 30 to 120  $\mu$ , all 4 samples having vessels within these limits. No references are available for the vessels of Orizaba jalap, but for Vera Cruz jalap, Moll and Janssonius<sup>3</sup> give the following:—R and T. 20  $\times$  25  $\mu$  up to 40  $\times$  50  $\mu$ , the length of the articulations 70 to 170  $\mu$ . A distinctive feature of the vessels of Brazilian jalap is the scalariform-reticulate thickening and the absence of vessels with rounded or oval bordered pits. This enables Brazilian jalap to be distinguished from Vera Cruz jalap and Orizaba jalap, both of which have vessels with rounded or oval bordered pits. Because of this qualitative difference no attempt was made to measure diameters of the vessels in the samples of Vera Cruz jalap and Orizaba jalap available.

Tracheids are present in all the 3 varieties of jalap. The dimensions of the tracheids of Brazilian jalap given by Farwell have been quoted above. No references are available for the dimensions of the tracheids of Vera Cruz jalap and Orizaba jalap and, in fact, no reference is made to their presence in descriptions of the histology of these two tubercles. In the samples examined there appeared to be only a few tracheids, most of the bundles consisting of vessels and fibre tracheids. The fibre tracheids are present in all the 3 varieties of jalap but reference is available only for those in Vera Cruz jalap. Moll and Janssonius<sup>3</sup> give the size as 100 to 170  $\mu$  long and 15 to 20  $\mu$  diameter. Measurements of the fibre tracheids of Brazilian jalap recorded in Part I are 480 to 750  $\mu$  long and 25 to 50  $\mu$  wide, and those observed for Orizaba jalap are 250 to 600  $\mu$  long and 15 to 50  $\mu$  wide. In all cases the fibre tracheids have oblique slit-like pits. As reported in Part I, fibres are absent from all the samples

of Brazilian jalap examined. It is probable, therefore, that the fibres referred to by Farwell are, in fact, fibre tracheids. Since fibres are absent also from Vera Cruz jalap, they occur only in Orizaba jalap where they are present in large numbers. No references to their sizes are available but observations made indicate that they have fairly thick walls with simple pits and measure from 300 to 1500  $\mu$  long and 10 to 30  $\mu$  wide.

*Stone Cells.* Stone cells are present in small numbers in the phelloderm of both Vera Cruz jalap and Orizaba jalap but were present only in Sample A of the Brazilian jalap. The recorded dimensions of the stone cells are:—Brazilian jalap (Sample A only), 40 to 160  $\mu$  long, 30 to 60  $\mu$  wide, cell wall 12 to 25  $\mu$  thick. Vera Cruz jalap, 50 to 150  $\mu$  long, 30 to 80  $\mu$  wide, cell wall 10 to 25  $\mu$  thick. Orizaba jalap, 80 to 175  $\mu$  long, 50 to 150  $\mu$  wide, cell wall 10 to 25  $\mu$  thick. In all cases the cells have branched simple pits.

Elongated stone cells are present only in Sample A of the Brazilian jalap. They measure up to 600  $\mu$  long and 60  $\mu$  wide and have lignified cell walls 8 to 12  $\mu$  thick. The pitting is simple but unbranched.

*Latex Cells.* In all the varieties of jalap, the latex was present in cells which occurred in longitudinal rows of 4 to 6 cells. The latex itself was soluble in solution of sodium hydroxide and chloral hydrate solution. It stained yellow with dilute solution of iodine, pink with solution of corallin soda and red with tincture of alkanet. There appeared to be no simple chemomicroscopical test which would differentiate between the latex of Brazilian jalap, Vera Cruz jalap and Orizaba jalap.

#### PHARMACOGNOSTICAL CHARACTERS

The pharmacognostical characters of the drug which are considered useful are crude fibre, ash, acid-insoluble ash, water-soluble extractive, alcohol (90 per cent.)-soluble extractive, resin, frothing power, appearance under ultra-violet light and appearance of simple chromatograms.

*Crude Fibre.* Crude fibre assays (Dutch process) were carried out as follows:—

1 g. of the powdered drug (No. 60 powder) was put into a 100-ml. porcelain dish and boiled with 50 ml. of 10 per cent. nitric acid, the ebullition being maintained for 1 minute. It was then filtered through fine linen at the pump and the residue washed with 100 ml. of boiling water. The residue was then returned to the dish by stretching the linen over the end of a glass funnel and scraping it off with a flexible metal spatula. It was then boiled with 50 ml. of 2.5 per cent. sodium hydroxide solution, ebullition being maintained for 1 minute. It was filtered through the same area of linen as before and washed with a further 100 ml. of boiling water. The fibre was then transferred to a tared watchglass and dried to constant weight at 100°C. The results obtained are as follows:—Brazilian jalap A 16.18, 15.23, 15.60, (12.78) 16.05 per cent.; B 9.62, 10.43, 10.05, 9.97 per cent.; C 9.25, 9.39, 9.64, 9.78 per cent.; D 10.28, 9.56, 9.89, 10.03 per cent. Similar determinations on Vera Cruz jalap gave 3.32, (4.41) 3.64, 3.68, 3.25 per cent. and on Orizaba jalap 15.43, 15.72 per cent.

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*Ash, acid-insoluble ash, water-soluble extractive, alcohol (90 per cent.) soluble extractive and resin.*

The results obtained by the methods of the British Pharmacopœia 1948 are shown in Table III.

TABLE III

			Ash	Acid-insoluble Ash	Water soluble extractive	Alcohol (90 per cent.) soluble extractive	Resin
			per cent.	per cent.	per cent.	per cent.	per cent.
Brazilian jalap	A	...	12·56, 12·73	2·04, 2·00	7·20	22·75	19·51
"	B	...	7·09, 7·13	1·03, 1·03	11·53	34·52	22·60
"	C	...	8·22, 8·18	2·24, 2·28	8·16	20·25	16·45
"	D	...	13·60, 13·56	2·64, 2·54	5·12	19·37	14·07
Vera Cruz jalap	...	...	4·03, 4·10		13·63	30·20	17·84
Orizaba jalap	...	...	10·17, 10·24		11·18	17·86	13·25

All the aqueous extracts were acid to litmus and the pH of the solutions were as follows:—Brazilian jalap A 5·62, B 4·40, C 4·83, D 4·85; Vera Cruz jalap 3·65, Orizaba jalap 3·81.

*Frothing Power.* It was observed that the water in which the samples of Brazilian jalap were soaked gave an appreciable froth when agitated, suggesting, of course, the presence of a saponin-like substance as a constituent. A simple test was devised whereby the samples could be examined for their frothing powers. Although it is known that neither Vera Cruz jalap or Orizaba jalap contains saponins, the test was also applied to these drugs for comparative purposes.

(a) *Powdered Drug.* About 0·1 g. of powdered drug was briskly shaken 20 times with 10 ml. of distilled water in a test-tube ( $5\frac{1}{2} \times \frac{1}{2}$  inch) (time of shaking = 3·5 sec. approx.). The test-tube was set aside and the behaviour of the froth noted. The results obtained are as follows:—

Brazilian jalap, (A), (B), (C), (D), considerable froth which still persisted after 15 minutes; Vera Cruz jalap, some frothing, which subsided almost immediately and which disappeared within 30 to 40 seconds; Orizaba jalap, some frothing which subsided fairly quickly, but which persisted as a ring of small air bubbles at the meniscus of the liquid for 10 to 12 minutes before finally disappearing.

(b) *Aqueous Extractive.* 10 ml. quantities of the aqueous extracts (5 g. to 100 ml. of distilled water) were shaken as described above, with similar results.

(c) *Alcoholic Extractive.* 1 ml. of the alcoholic extractive (10 g. to 100 ml. of alcohol (90 per cent.)) was diluted to 10 ml. with distilled water and shaken as described above. Precipitation of the resinous matter, particularly in the case of Vera Cruz jalap and Orizaba jalap interfered with the delicacy of the test since small particles of resin held air bubbles enclosed in thin films of liquid in the upper part of the test tube. In the case of Brazilian jalap the froth persisted for more than 15 minutes, but with the other two varieties no consistent results

could be obtained, though the froth had usually subsided within 5 or 6 minutes.

The results obtained by this simple frothing test suggests a very close relationship between the 4 samples of Brazilian jalap, and the test itself offers a quick and reliable method of distinguishing between Brazilian jalap and Vera Cruz jalap or Orizaba jalap when in the powdered condition. The presence of a saponin-like body in a Convolvulaceous root is unusual, though Kassner<sup>4</sup> reported the presence of saponins in a number of convolvulaceous seeds. Dragendorff<sup>5</sup> records the presence of a saponin in the root of the New South Wales *Ipomæa maritima* R. Br. (*Convolvulus brasiliensis*), and it is interesting to note that *Convolvulus brasiliensis* Linn is one of the botanical names of Brazilian jalap given by Tschirch<sup>6</sup>. However, examination of the root of this plant which is now known as *Ipomæa pes-caprae* Roth. failed to confirm the presence of saponins.

*Examination with Screened Ultra-violet Light.* Both Vera Cruz jalap and Orizaba jalap contain  $\beta$ -methylæsculetin which gives a blue fluorescence in alkaline solution, but there is no information available regarding the presence or absence of this substance in Brazilian jalap. In the endeavour to find a quick and reliable method of distinguishing between Brazilian jalap and the other two varieties, and still in search

TABLE IV  
EXAMINATION UNDER ULTRA-VIOLET LIGHT

Variety of Jalap	Aqueous* Extract	Alcohol (90 per cent.) Extract	Ether Extract	Chloroform Extract	Acetone Extract
Brazilian A ... ..	Very faint blue	Intense bright blue	Practically no fluorescence	Pale blue	Purplish blue
„ B ... ..	Very faint blue	Extremely intense deep blue	Very faint blue	Pale blue	Pale blue
„ C ... ..	Very faint blue	Intense whitish blue	Pale purplish blue	Pale purplish blue	Pale whitish blue
„ D ... ..	Very faint blue	Intense whitish blue	Pale purplish blue	Pale purplish blue	Pale whitish blue
Vera Cruz ... ..	Pale blue	Bright blue	Pale purplish blue	Pale purplish blue	Pale whitish blue
Orizaba ... ..	Deep blue	Intense whitish blue	Bright purplish blue	Bright purplish blue	Intense blue
$\beta$ -methyl æsculetin ...	Deep blue	Intense whitish blue	Bright purplish blue	Bright purplish blue	Intense blue

\* pH of aqueous extractives similar to those given in a previous paragraph.

of evidence which might differentiate between the 4 samples of Brazilian jalap, it was decided to examine various extractives of these drugs under ultra-violet light. Separate quantities of 10 g. of the powdered drug were extracted in a Soxhlet extractor with the following solvents:—water, alcohol (90 per cent.), ether, chloroform and acetone and the volume adjusted to 100 ml. One drop of each extractive was placed upon a Whatman No. 1 filter paper, allowed to dry and then examined with screened

ultra-violet light (wave-length peak 3660Å). The results obtained are shown in Table IV.

When exposed to ammonia vapour and examined by screened ultra-violet light the appearance of some of the spots changed as follows:—

$\beta$ -methyl *æsculetin*—the colour changed rapidly from blue to yellowish green with all 5 solvents.

Brazilian jalap A, C, D; the colour of the aqueous and alcoholic extractives changed slowly to be distinctly green in colour. There was no change with the other extractives. B; the colour of the aqueous and alcoholic extractives changed quite quickly to a distinct yellowish green.

Vera Cruz jalap and Orizaba jalap; the aqueous and alcoholic extractives quickly turned yellowish green, but there was no change with the other extractives.

The slight differences noted between the appearances of the extractives of the 4 samples of Brazilian jalap when examined with ultra-violet light as described are not sufficient to infer any difference between the drugs, and although the differences between the 3 varieties of jalap are quite sharp they are not sufficiently well defined to enable the test to be used in order to distinguish between them. The results obtained, however, suggest the presence of a second fluorescent substance in all three jalaps. It is clear that  $\beta$ -methyl *æsculetin* is also present in Brazilian jalap, but in much smaller amount than in Vera Cruz or Orizaba jalap.

*Elementary Chromatograms.* Disc chromatograms were prepared by the method described by T. I. Williams<sup>7</sup>. The adsorbent was heavy magnesium oxide and the chromatogram was developed with the same solvent as that used in preparing the extractive. The extracts were made with: (1) water made alkaline with dilute solution of ammonia, (2) alcohol (90 per cent.), (3) ether, (4) chloroform, and (5) acetone.

Examined in daylight the zones were very pale and except in the case of the chromatogram prepared from the ethereal extractive not sufficiently well defined to enable the different varieties to be distinguished.

Examined in screened ultra-violet light, all the chromatograms prepared exhibited zones which were well defined and sufficiently distinctive to be useful diagnostically.

The chromatograms obtained from the 4 samples of Brazilian jalap indicated that they were almost identical, the differences being in the width of the zone or intensity of the colour only. The differences between the chromatograms of the 3 varieties of jalap were so pronounced as to distinguish very clearly between them. The appearance of the chromatograms also support the suggestion that there are constituents other than  $\beta$ -methyl *æsculetin* which give a blue fluorescence. Work on the chromatographic examination of the 3 varieties of jalap, particularly the resins, is being continued.

#### SUMMARY AND CONCLUSIONS

The further histological examination and the pharmacognostical studies of the 4 samples of commercial Brazilian jalap indicate that the tubercles are so similar as to be considered genuine samples of Brazilian jalap.



No evidence was obtained which would confirm whether the tubercles were from identical or different biological sources.

The characters which are common to all four samples of Brazilian jalap are:—

- (1) General morphological characters.
- (2) The absence of vessels with round or oval bordered pits. The vessels have reticulate scalariform thickening.
- (3) The sizes and appearance of the tracheids and fibre tracheids. The absence of true fibres.
- (4) The sizes of the crystals of calcium oxalate.
- (5) The number of cork cells per sq. mm. surface area.
- (6) The constituents as indicated by examination of disc chromatograms prepared from extracts of the drug with various solvents using heavy magnesium oxide as adsorbent.
- (7) The presence of a saponin-like constituent which produces a permanent froth when the powdered drug is shaken with water.

The main differences between the 4 samples are histological and were described in Part I.

Comparisons of the characters common to all the 4 samples of Brazilian jalap and the same characters of Vera Cruz and Orizaba jalap indicate well-defined differences between some of the characters which are sufficient to enable the three varieties to be distinguished from each other when in the powdered condition.

The features which are most useful for distinguishing between Brazilian jalap, Vera Cruz jalap and Orizaba jalap are given in Table V.

TABLE V  
DISTINGUISHING FEATURES OF BRAZILIAN, VERA CRUZ AND ORIZABA JALAP

	Brazilian jalap	Vera Cruz jalap	Orizaba jalap
Presence or absence of saponin-like constituents	Present	Absent	Absent
Type of pits in the vessels	Elongated, giving the cell wall a scalariform-reticulate appearance. Absence of round or oval bordered pits	Round or oval bordered	Round or oval bordered
Presence or absence of true fibres	Absent	Absent	Lignified fibres with fairly thick walls
Maximum size of calcium-oxalate crystals	40 $\mu$	30 $\mu$	45 $\mu$

Comparative examination of disc chromatograms in ultra-violet light prepared from various extractives, using heavy magnesium oxide as the adsorbent.

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