Keto Fatty Acids from *Cuspidaria pterocarpa* Seed Oil[1]

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ABSTRACT

The seed oil of *Cuspidaria pterocarpa* contains three keto fatty acids with unusually long carbon chains: 15-oxo-*cis*-18-tetracosenoic (5.4%), 17-oxo-*cis*-20-hexacosenoic (13.4%), and 19-oxo-*cis*-22-octacosenoic (3.3%) acids. These acids were isolated by countercurrent distribution of the corresponding methyl esters. Their structures were established by oxidative degradation, by reduction to known compounds, and by nuclear magnetic resonance and infrared spectra.

INTRODUCTION

FATTY ACIDS CONTAINING ketone functions are rarities in natural lipids of plant origin. Apparently, the only ones whose complete structures have been recorded in the literature previously are 4-oxo-*cis*-9,*trans*-11,*trans*-13-octadecatrienoic (α-licanic) and 9-oxo-*trans*-10, *trans*-12-octadecadienoic acids. The former is a major constituent of Brazilian oiticica oil (1). The latter of these two was found recently as a minor constituent of *Dimorphotheca* seed oil (2).

Preliminary investigation of seed oil of *Cuspidaria pterocarpa* (Cham.) DC. (family Bignoniaceae) suggested the presence of three keto fatty acids. The present paper describes the isolation and structural elucidation of these keto acids. The structure of one has been reported in a preliminary communication (3).

EXPERIMENTAL PROCEDURES AND RESULTS

General Methods

Esterifications and transesterifications were carried out as follows, except where otherwise specified: Samples were refluxed 1 hr in a large excess of methanol containing 1% sulfuric acid (v/v). In each case, resulting mixtures were diluted with water, chilled in an ice bath, and then extracted repeatedly with petroleum ether (bp 30C–60C). Combined extracts were dried over sodium sulfate and evaporated in vacuo.

Gas-liquid chromatographic (GLC) analyses were carried out exactly as described by Miwa and co-workers (4), including the use of apparatus and detector described.

Thin-layer chromatography (TLC) was performed on plates coated with Silica Gel G (according to Stahl) with the solvent system hexane-diethyl ether-acetic acid (80:20:1).

Infrared (IR) spectra were determined with a Perkin-Elmer Model 337 instrument, on 1% solutions in carbon tetrachloride. Nuclear magnetic resonance (NMR) spectra were obtained with a Varian A-60 spectrometer on 1% deuteriochloroform solutions.

Melting points were determined with a Fisher-Johns block and are uncorrected.

Preparation of Mixed Methyl Esters

Coarsely ground seeds of *Cuspidaria pterocarpa* (Cham.) DC. (13.87 g) were extracted overnight in a Soxhlet apparatus with petroleum ether (bp 30C–60C). Upon evaporation of solvent, 7.87 g of oil was obtained. Its IR spectrum showed a double carbonyl peak having a strong maximum at 1740 cm^{-1} (ester) and a weaker one at 1720 cm^{-1} (ketone).

A 3.21 g portion of the oil was converted to a mixture of methyl esters by transesterification; a yield of 3.12 g of methyl esters was obtained. Their IR spectrum was very similar to that of the oil in the carbonyl region. The esters had the following composition (expressed as wt %): C_{16}, 2.6; C_{18}, 68.3; C_{20}, 5.2; C_{22}, 0.9; C_{24} keto, 5.4; C_{26} keto, 13.4; and C_{28} keto, 3.3. It became apparent from the amount of C_{28} keto ester isolated subsequently that the value obtained by GLC for this compound was considerably too low. Consequently, the weight percentages indicated here for the various components have been corrected on the basis of estimates of the amounts of keto esters in fractions obtained by countercurrent distribution.

In order to determine whether acid-catalyzed transesterification appreciably altered the composition of *Cuspidaria* oil, methyl esters also were prepared by two other procedures: *Cuspidaria* oil was saponified by stirring 24 hr with 1 *N* ethanolic potassium hydroxide at ambient temperature under a nitrogen atmosphere. A portion of the free acids thus obtained was esterified with diazomethane. Another portion was esterified with 1% methanolic sulfuric acid. The three ester preparations were essentially identical as judged by TLC, GLC, and IR. Only 1.4% of unsaponifi-

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ables was found in the oil. Consequently, the acid-catalyzed transesterification method was chosen for large-scale preparation of methyl esters from *Cuspidaria* oil because of its simplicity.

Countercurrent Distribution of Methyl Esters

Countercurrent distribution (CCD) of *Cuspidaria* mixed methyl esters was carried out with an acetonitrile-hexane system by the general procedure of Scholfield et al. (5). An 11.01 g portion of the mixed esters was divided among the first 6 tubes of a 200-tube Craig-Post apparatus. Ten milliliters of upper phase and 40 ml of lower phase were used throughout the distribution. After the 200 fundamental transfers had been completed, upper phases were decanted into a fraction collector; four transfers were combined in each tube. Apparently the entire starting sample emerged from the apparatus by the time 600 transfers had been applied. The three keto esters formed three discrete peaks, satisfactorily resolved from one another. Unfortunately, however, these three coincided with peaks formed by esters of common fatty acids that were present in larger amounts—oleate, linoleate, and linolenate. Results of GLC analyses of esters from selected CCD tubes are given in Table I. Concentrates of the individual keto esters suitable for isolation of more highly purified compounds for characterization were obtained by combining contents of appropriate tubes.

TABLE I

Composition of CCD Fractions
(Results estimated by GLC and expressed as area percent)

Tube number[a]	Transfers[b]	% Keto esters[c]	% Non-Keto esters[d]
20	280	0.7 C_{28}	11.2 C_{16}, 14.2 C_{18}S, 38.5 C_{20}, others
25	300	9.6 C_{28}	13.6 C_{16}S, 72.2 C_{18} (Mostly C_{18}I), 3.7 C_{20}
30	320	16.7 C_{28}, 1.0 C_{26}	16.3 C_{16}S, 73.9 C_{18} (Mostly C_{18}I), 3.2 C_{20}
35	340	13.4 C_{28}, 18.9 C_{26}	54.8 C_{18}I, 31.4 C_{18}II
40	360	44.0 C_{26}	55.3 C_{18}II
50	400	32.4 C_{26}	66.4 C_{18}II
55	420	30.8 C_{26}, 2.1 C_{24}	66.0 C_{18}II
60	440	12.2 C_{26}, 31.8 C_{24}	51.7 C_{18}II + C_{18}III
65	460	0.3 C_{26}, 44.9 C_{24}	54.3 C_{18}III
70	480	36.9 C_{24}	62.8 C_{18}III
80	520	22.8 C_{24}	77.3 C_{18}III
90	560	16.5 C_{24}	83.0 C_{18}III

[a] Numbers of tubes used to collect decanted upper phases. Four were combined in each tube.

[b] Number of transfers completed when upper phase was introduced into the tube indicated.

[c] The indicated percentages are subject to error, especially in the case of the C_{28} keto ester (see text).

[d] Where indicated the symbols have the following significance: S = Saturated; I = one double bond; II = two double bonds; III = three double bonds.

Isolation and Preliminary Characterization of Keto Esters

The individual keto esters were isolated by low-temperature crystallization. The hexane solutions of mixed esters that resulted from combining CCD fractions were reduced considerably in volume and then stored at −18C for 3 days. The resulting mixtures were then filtered with conventional Büchner funnels at the same temperature; collected products were washed with a small amount of cold solvent.

C_{26}-Keto Ester (Ib). Low-temperature crystallization of material from CCD tubes 41–54 afforded 1.12 g of C_{26}-keto ester (Ib), mp 41.5–43.5C. When 0.049 g of this was recrystallized from methanol, 0.018 g was provided, mp 44–45C. Ib had strong IR maxima at 1720 and 1745 cm^{-1}. With platinum oxide catalyst, Ib consumed 1.1 moles of hydrogen in ethanol solution. The NMR spectrum of Ib is summarized in Table II and Figure 1.

Anal. Calcd. for $C_{27}H_{50}O_3$ (422.67): C, 76.7; H, 11.9. Found: C, 76.6; H, 12.0.

C_{24}-Keto Ester (Ia). Low-temperature crystallization of esters from CCD tubes 66–79 (Table I) provided 0.349 g of Ia, mp 35–37.5C. Recrystallization of 0.080 g of this product from aqueous methanol afforded 0.061 g of Ia, mp 40.0–40.5C. Its NMR spectrum is summarized in Table II.

Anal. Calcd. for $C_{25}H_{46}O_3$ (394.62): C, 76.1; H, 11.8. Found: C, 75.6; H, 11.5.

C_{28}-Keto Ester (Ic). An 0.214 g portion of Ic was obtained by low-temperature crystallization of esters from CCD tubes 21–29 (Table I), mp 47C–49C. Recrystallization of 0.035 g of this product from methanol provided 0.033 g of Ic having mp 50.0–51.5C. Its NMR spectrum is summarized in Table II.

Anal. Calcd. for $C_{29}H_{54}O_3$ (450.72): C, 77.3; H, 12.1. Found: C, 76.8; H, 11.9.

Sodium Borohydride Reduction of C_{26}-Keto Ester

An 0.200 g portion of Ib was dissolved in 30 ml of methanol; a solution of 0.6 g of sodium

TABLE II

NMR Spectra of *Cuspidaria* Keto Esters

Assignment	τ-Value ppm	Number of protons Ia	Ib	Ic
CH_3, terminal	9.12	3	3	3
CH_2, shielded	8.73	28	32	36
CH_2, *a* to carbonyl or double bond	7.62	10	10	10
OCH_3	6.33	3	3	3
Olefinic H	4.63	2	2	2
Total protons		46	50	54

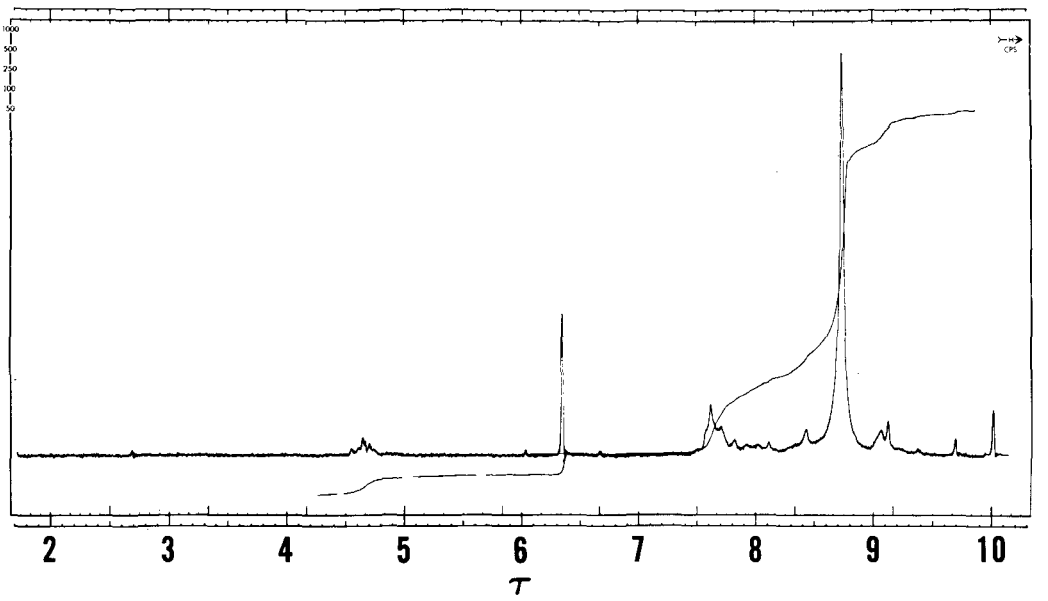

FIG. 1. NMR spectrum of C_{26}-keto ester (Ib).

borohydride in 20 ml of methanol was added. After the initial vigorous reaction had subsided, the mixture was refluxed 4.5 hr under a nitrogen atmosphere, acidified with hydrochloric acid and then extracted repeatedly with petroleum ether (bp 30–60C). Combined extracts were dried over sodium sulfate, and then evaporated. The resulting residue was reesterified since IR indicated some hydrolysis of the carbomethoxy grouping. The crude product, 0.211 g, had mp 36–42C. Two recrystallizations from aqueous methanol afforded 0.018 g of ester IIb, mp 46–47C. The IR spectrum of IIb showed maxima at 1740 cm^{-1} (ester) and 3635 cm^{-1} (OH).

Anal. Calcd. for $C_{27}H_{52}O_3$ (424.69): C, 76.4; H, 12.3. Found: C, 76.5; H, 12.5.

Reductive Deoxygenation of C_{26}-Keto Ester

An 0.106 g portion of Ib was hydrogenated at ambient temperature in 20 ml of methanol with platinum oxide catalyst. The saturated product, IIIb, was recrystallized twice from methanol; 0.039 g having mp 70.0–70.5C was obtained.

Anal. Calcd. for $C_{27}H_{52}O_3$ (424.69): C, 76.4; H, 12.3. Found: C, 76.7; H, 13.0.

Saturated keto ester IIIb was reduced with sodium borohydride essentially as was Ib. The reduction product was subjected to hydrogen iodide-phosphorus reduction by the general procedure of Meakins and Swindells (6). An 0.071 g portion of saturated hydroxy ester was refluxed 18 hr with 5 ml of hydriodic acid and 50 mg of red phosphorus. The mixture was diluted with water and extracted repeatedly with petroleum ether. Combined extracts, after having been dried with sodium sulfate, were evaporated. The oily residue was refluxed 4 hr with 0.20 g of granular zinc, 1 ml of hydrochloric acid, and 5 ml of methanol. The reduction product (IVb) was isolated by extraction with petroleum ether. Evaporation of combined extracts afforded 0.018 g having mp 57–59C. Two recrystallizations from methanol provided a sample of IVb having mp and mixed mp 61.5–62.0C (lit. mp 62C (7)). A mixture of ester IVb and an authentic specimen of methyl hexacosanoate was not resolved during GLC.

Permanganate-Periodate Oxidation of Keto Esters

Each of the three esters was oxidatively cleaved by von Rudloff's permanganate-periodate method, specifically the modification in which *t*-butyl alcohol is used as a cosolvent (8). Each yielded hexanoic acid as the only monobasic acid cleavage product, as determined by GLC.

An 0.126 g portion of Ib yielded 0.141 g of oxidation products. Two recrystallizations from benzene afforded 0.069 g of γ-keto acid Vb, mp 123–124C. Vb was obtained as a free acid because the strongly alkaline conditions during the workup hydrolyzed the ester grouping.

Anal. Calcd. for $C_{20}H_{36}O_5$ (356.49): C, 67.4; H, 10.2. Found C, 67.4; H, 10.3.

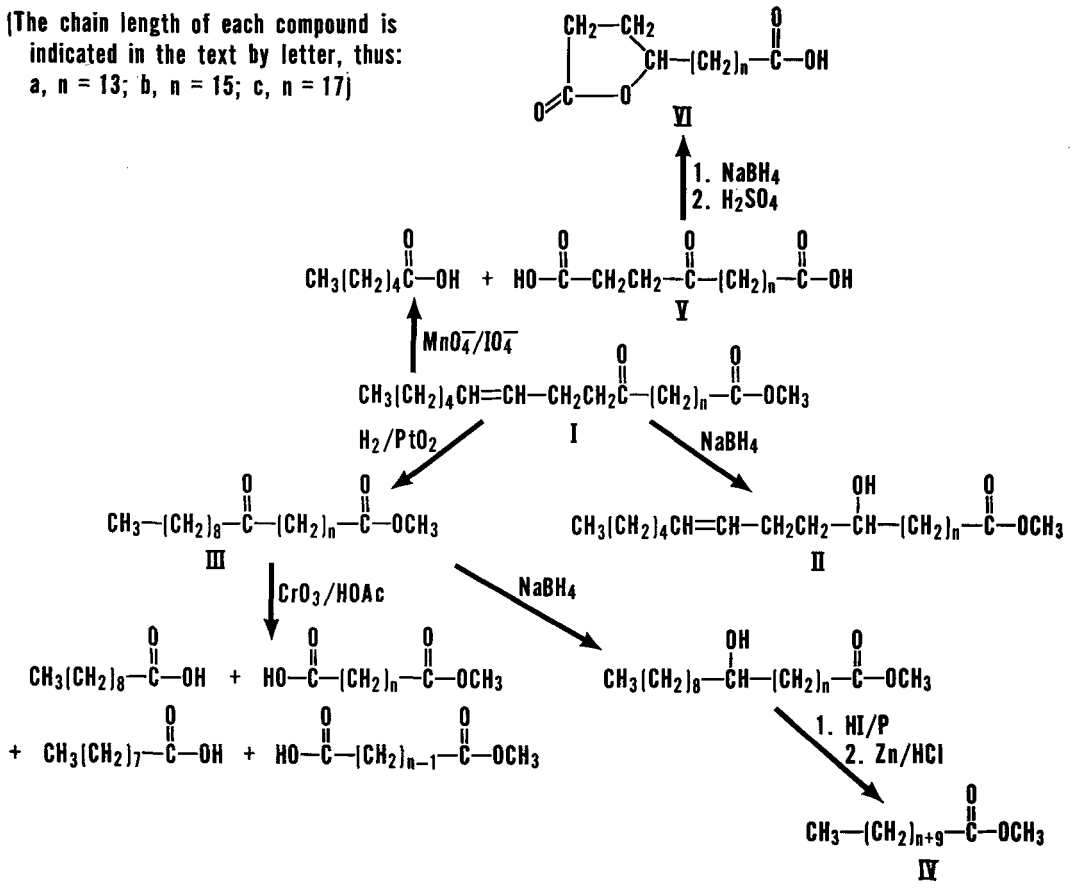

FIG. 2. Flow sheet and reaction scheme.

Vb was converted to a methyl ester for determination of IR spectrum; this showed maxima at 1720 (ketone) and 1745 cm^{-1} (ester).

An 0.137 g portion of Ia yielded 0.143 g of oxidation products. Two recrystallizations from benzene provided 0.073 g of 4-oxooctadecanedioic acid (Va), mp 122–123C. Elementary analyses of Va were not obtained owing to the accidental destruction of the analytical sample.

Oxidation of 0.088 g of C_{28}-keto ester provided 0.065 g of oxidation product. This product was recrystallized from benzene-hexane, and 0.034 g of 4-oxodocosanedioic acid (Vc), mp 119.5–121.0C, was obtained.

Anal. Calcd. for $C_{22}H_{40}O_5$ (384.54): C, 68.7; H, 10.5. Found: C, 68.2; H, 10.4.

Conversion of γ-Keto Acids to γ-Lactones

Each of the three γ-keto acids (Va, Vb, and Vc) was reduced with sodium borohydride in methanol. The reduction products were lactonized by treatment with aqueous mineral acid. The following example is typical of the procedure used:

Sodium borohydride (0.270 g) dissolved in 15 ml of methanol was added to 0.036 g of Vb dissolved in 10 ml of methanol. After the vigorous initial reaction subsided, the mixture was refluxed 2 hr, and then diluted with water, acidified with hydrochloric acid, and extracted with ethyl ether. The crude product thus isolated was refluxed 4 hr with 1% aqueous sulfuric acid. Extraction with ethyl ether and evaporation provided 0.032 g of 4-hydroxydocosanedioic acid lactone (VIb). Recrystallization from benzene-hexane provided 0.023 g of VIb, mp 95.0–95.5C. The IR spectrum of VIb showed maxima at 1720 cm^{-1} (carboxylic acid) and 1775 cm^{-1} (γ-lactone.)

Anal. Calcd. for $C_{20}H_{36}O_4$ (340.49): C, 70.5; H, 10.7. Found: C, 70.2; H, 10.6.

Chromium Trioxide Oxidation of Keto Esters

Each of the three keto esters—Ia, Ib, and Ic—was hydrogenated preparatively, and then

cleaved with chromium trioxide-acetic acid as described by Meakins and Swindells (6) with some modifications. The following will serve to illustrate:

Ester IIIb (0.028 g) was stirred 1 hr at ambient temperature with a solution of 0.35 g of chromium trioxide in 2.5 ml of acetic acid and 0.3 ml of water. Then the mixture was diluted with water and extracted with ethyl ether. The combined extracts were dried over sodium sulfate, and then distilled with a short Vigreux column to remove solvent. The residue was esterified and examined by GLC. C_9 and C_{10} monobasic esters were found together with C_{16} and C_{17} dibasic esters. A considerable number of other degradation products were also present; these appeared to be derived mainly from the dicarboxylic acids.

IIIa and IIIc likewise yielded nonanoate and decanoate as degradation products when treated in the manner just outlined. In addition, the C_{24} ester yielded C_{14} and C_{15} dicarboxylic acids, and the C_{28} ester afforded C_{18} and C_{19} dicarboxylic acids as cleavage fragments.

DISCUSSION

Ester Ib contains an unconjugated carbonyl group, since its IR spectrum shows a maximum at 1720 cm^{-1} in addition to the one due to the ester group (1745 cm^{-1}). The reduction of keto ester Ib to methyl hexacosanoate shows that Ib must possess a normal C_{26} carbon skeleton. The NMR spectrum of Ib is in full accord with this conclusion since it shows only one C-methyl group. Ib must contain one double bond, since it consumes one molar equivalent of hydrogen and yields two acidic fragments when oxidized with permanganate-periodate. The IR spectrum of Ib shows that this double bond is *cis*. Its NMR spectrum (Fig. 2) shows but two olefinic protons (4.65τ), and thus indicates but one double bond. The formation of hexanoic acid as one product of permanganate-periodate oxidation of Ib indicates that the double bond must be six carbons from the end of the chain—i.e. in the Δ^{20} position. The other fragment from the oxidative degradation, keto acid Vb, must be a γ-keto acid since it can be reduced to a γ-lactone. This observation indicates that the double bond and oxygen function of Ib must have a 1,4-relationship. Our inference is supported by the results of chromium trioxide oxidation of saturated ester IIIb, since both lines of evidence place the oxygen function at C-17. As expected, Ib underwent facile reduction to unsaturated hydroxy ester IIb in the presence of sodium borohydride. Thus Ib must be an ester of 17-oxo-*cis*-20-hexacosenoic acid.

The structural elucidation of the C_{24} and C_{28} keto acids (Ia and Ic) followed the same general scheme. Oxidative cleavages showed that each contains one double bond and that it has the same position relative to the keto group and the terminal methyl group as does the double bond in Ib. The NMR spectra of the three keto esters are very similar and indicate that all have only one C-methyl group and one olefinic bond. All three spectra are characterized by a signal at 7.62τ. According to Hopkins (9) this chemical shift is associated with methylene protons in the grouping $-CH=CH-CH_2CH_2-\overset{O}{\overset{\|}{C}}-$. The NMR spectra of the three differ only in the number of shielded methylene protons found (8.73τ). Thus Ia must be 15-oxo-*cis*-18-tetracosenoic acid, and Ic is 19-oxo-*cis*-22-octacosenoic acid.

The smooth oxidation of keto esters Ia, Ib and Ic by permanganate-periodate to afford hexanoic acid and γ-keto acids could not have been anticipated with certainty, since cases have been encountered in which the formally expected cleavage products of long-chain compounds have been destroyed by this reagent (10,11,12). von Rudloff recently reported that 2,5-hexanedione is oxidized appreciably on prolonged treatment with permanganate-periodate (13).

The chromium trioxide-acetic acid cleavage method appears to be less satisfactory for these C_{24} to C_{28} acids than for those in the C_{18} to C_{20} range. Undesired degradation products were more numerous and were formed in larger amounts than with shorter-chain acids.

The cause of the error in values for the C_{28} keto ester in GLC analyses of mixtures and of methyl esters is not known; it may be low detector response and/or nonquantitative elution from the column. Since GLC analyses were applied to CCD fractions, the values in Table I are unavoidably subject to some error. At present, we do not have a satisfactory method for determining the C_{28} keto ester in lipid mixtures.

The keto acids of *Cuspidaria pterocarpa* seed oil combine two features rarely found in lipids of higher plants—ketone functions together with chain lengths longer than C_{22}. Their biogenetic origin presents an interesting problem for speculation. The co-occurrence of these homologs, differing only in the number of methylene groups between the keto and carboxyl functions, suggests that the three have the same direct

precursor. However, either the double bond or the carbonyl function could be introduced after the carbon chain already had been elaborated. There is now evidence, incidentally, that oleic is the direct precursor of ricinoleic acid (14,15).

Seed oils of comparatively few species in the family Bignoniaceae have been studied. Some that have been examined are sources of unusual acids; many of these have conjugated unsaturation (16,17). No consistent pattern of fatty acid composition of seed oils is apparent in this family.

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