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BRANCHED DEOXYALDARIC ACIDS FROM ALKALINE DEGRADATION OF CARBOHYDRATES: STRUCTURE DETERMINATION BY MASS SPECTROMETRY OF TRIMETHYLSILYL DERIVATIVES

GÖRAN PETERSSON

Department of Engineering Chemistry, Chalmers Tekniska Högskola, Göteborg (Sweden)

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ABSTRACT

The deoxyaldaric acids corresponding in structure to the 3-deoxy-2-*C*-(hydroxymethyl)aldonic (isosaccharinic) acids have been identified as products of treatment of various carbohydrates with alkali and oxygen–alkali. The structures of the acids were determined from the mass spectra of their Me_3Si derivatives on the basis of previously known, specific fragmentation reactions. The g.l.c.–m.s. technique was used, and g.l.c. retention data are given. The identified species are 2-deoxy-3-*C*-(hydroxymethyl)tetraric, 3-deoxy-2-*C*-hydroxymethyl-*erythro*-pentaric, 3-deoxy-2-*C*-hydroxymethyl-*threo*-pentaric, 2-methyltartronic, 2-(2-hydroxyethyl)tartronic, and 2-(2,3-dihydroxypropyl)tartronic acids. Their formation from 4-*O*-substituted uronic and ulosonic acids is briefly discussed.

INTRODUCTION

The deoxyaldonic (saccharinic) acids and their formation by the alkaline degradation of carbohydrates have been extensively studied[1–3]. By contrast, the deoxyaldaric acids are largely unknown, although they should be formed from many types of acidic carbohydrates by similar reactions. The two diastereomeric 3-deoxy-2-*C*-(hydroxymethyl)pentaric acids obtained by alkaline degradation of alginates[4] constitute an exception. Clearly, the lack of adequate analytical methods has hampered research in this area.

The preparation of acyclic trimethylsilyl (Me_3Si) derivatives of hydroxy acids and the application of g.l.c.–m.s. to the separation and identification of these derivatives[5] offer powerful new tools for the study of aldaric and deoxyaldaric acids. In combination with ion-exchange fractionation[6] and chromatography[7], complex mixtures can be analysed even for trace components, as demonstrated in a recent study of the oxygen–alkali treatment of cellulose[6]. The present paper describes the application of m.s. in the structure elucidation of branched deoxyaldaric acids, which were found to be prominent products from the degradation of carbohydrates in both oxygen-free and oxygen-containing aqueous alkali[6,8].

In accordance with aldaric acid nomenclature, acids derived from malic acid are termed 2-deoxytetraric acids in this work.

STRUCTURAL ANALYSIS

The potential of electron-impact m.s. for the determination of new structures has probably not yet been fully recognized. Predictable specific fragmentations make this method very useful for carbohydrate derivatives. Its application in this work is based on previous detailed studies of acyclic Me_3Si derivatives of aldonic and deoxyaldonic acids[9], and of unbranched aldaric and deoxyaldaric acids of synthetic origin[10]. The Me_3Si derivatives of the acids were prepared from their sodium salts as in earlier work[5], and an LKB 9000 g.l.c.–m.s. instrument was employed as described previously[10].

Structurally diagnostic fragmentations. — Three types of fragmentations are particularly valuable for structural analysis.

The best-known of these is the decomposition of the molecular ion of mass M by the loss of a Me group to give M–15 ions. Whereas M peaks are very small or absent, a relative intensity of 5–10% is usually observed at 70 eV for the M−15 peaks for C_3 through C_6 hydroxy-dicarboxylic acids[10]. The M−15 peak is easily recognized as the one with the highest, odd mass-number in the spectrum and gives the M value.

A second, structure-specific fragmentation is the McLafferty-type rearrangement of a Me_3Si group in Me_3Si derivatives of α,β-dihydroxycarbonyl compounds. This rearrangement has been thoroughly investigated for various types of carbohydrate-related compounds[11], and safe predictions of its course for branched deoxyaldaric acids can be made (Fig. 1). The rearrangement ions of high mass are often of moderate or low intensity because of further fragmentation by allylic cleavage. Thus, the M−30 and M−44 ions shown in Fig. 1 may decompose to *m/e* 305 ions by the loss of R^1 and R^2. However, peaks due to the odd-electron rearrangement ions are easily recognized, even if they are of low intensity, because they appear at even mass numbers. Branched acids with an additional $OSiMe_3$ group linked to the carbon atom adjacent to R^1 and R^2 would give rise to abundant *m/e* 394 and 408 ions (in preference to unstable M−30 or M−44 ions) by rearrangement of this $SiMe_3$ group. It should also be noted that the strong suppression of the rearrangement, if the $OSiMe_3$ group at C-2 is lacking, can be used for structural conclusions.

The third useful fragmentation is C–C-cleavage promoted by ether-type oxygen atoms (α-cleavage). The resulting oxonium ions are abundant and characteristic in the spectra of acyclic Me_3Si derivatives of aldonic and aldaric acids, and their formation and further fragmentation were therefore studied for these compounds[9,10]. The corresponding peaks often indicate the presence of a certain structural moiety, and they are particularly useful as evidence for the position of "deoxy groups". In structural analysis, it should be noted that the polar ester-carbonyl group counteracts the formation of α-cleavage ions with charge retention at the carbon atom adjacent

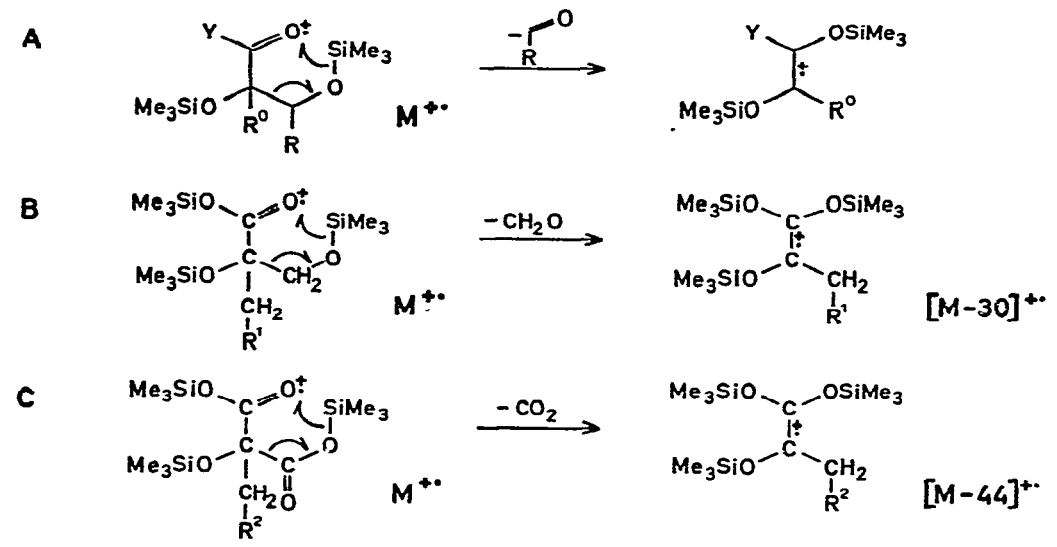


Fig. 1. Specific m.s. McLafferty-type rearrangement of a Me_3Si group in Me_3Si derivatives. A, general formulation; B, formulation for 2-substituted glyceric acids; C, formulation for 2-substituted tartronic acids.

to the carbonyl group. It should also be noted that there is no reliable relationship between the common CH_2OSiMe_3 group and the abundance of the *m/e* 103 ion, as this ion can be formed by rearrangement and also easily fragments further.

The fragmentations discussed were used for the identification of branched deoxyaldaric acids as described below. The ions used as proof of structure are marked in bold-face in the reproduced spectra. The masses of other ions formed by analogy with previously described fragmentations[9,10] are also indicated, but these ions are not further discussed.

2-Methyltartronic acid. — The spectrum in Fig. 2 corresponds to a component of the dicarboxylic acid fraction obtained from oxygen–alkali treatment of hydrocellulose[6]. The peak at *m/e* 335 is ascribed to M − 15 ions and indicates a C_4 deoxy-

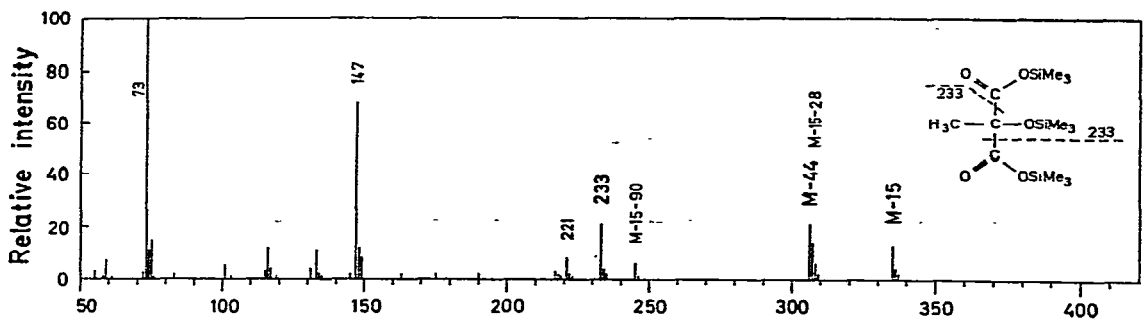
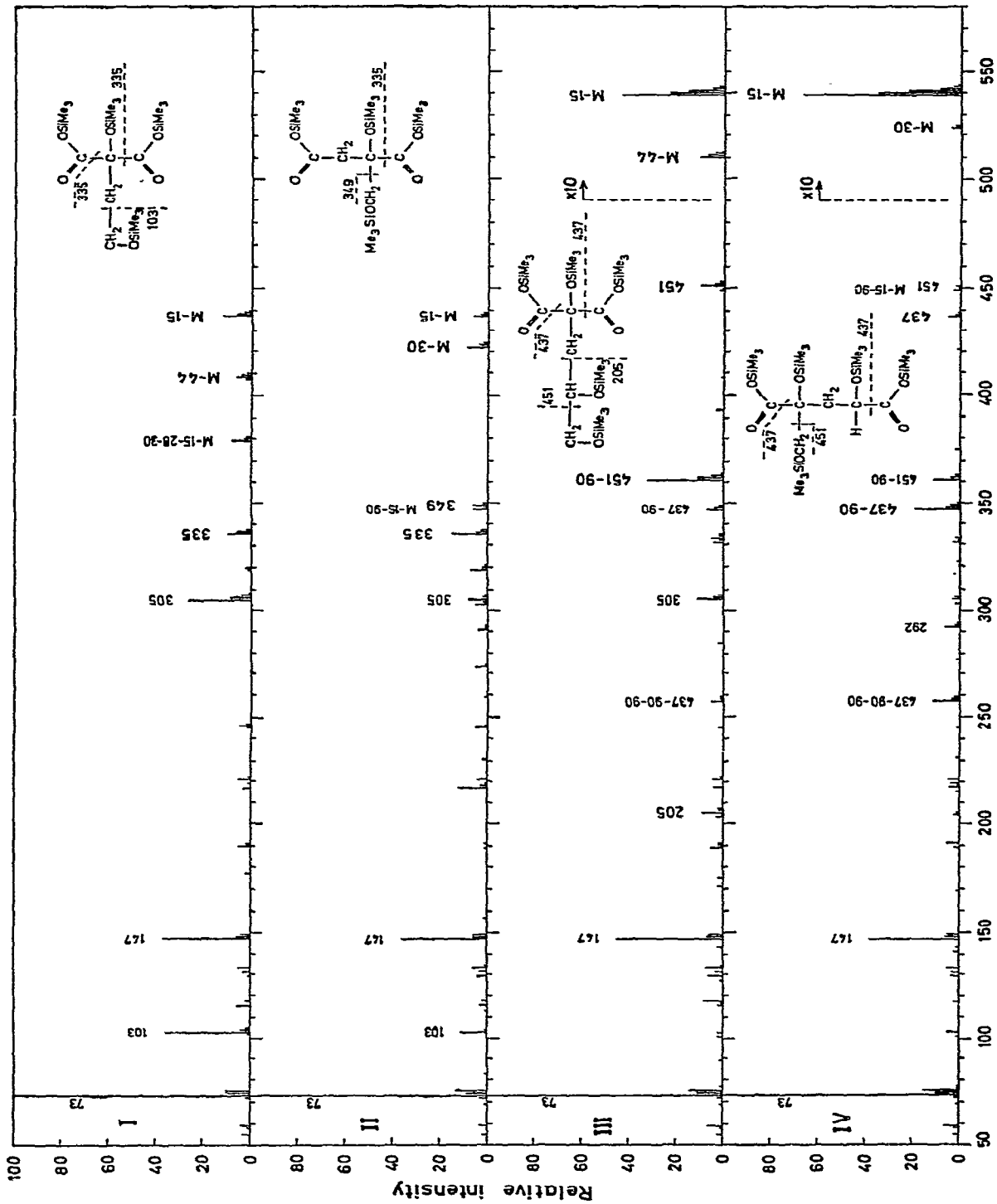


Fig. 2. Mass spectrum at 70 eV of the Me_3Si derivative of 2-methyltartronic acid.


Relative intensity
I
II
III
IV
M-15
M-30
M-44
M-15-28-30
M-15-90
M-30-90
M-44-90
147
103
73
305
335
349
451
437
205
292
x10

aldaric acid (mol. wt., 350). The intense M−44 peak is compatible with 2-methyltartronic acid (Fig. 1C), but not with the isomeric acids. A comparison with the fragmentation of the tartronic and deoxytetraric (malic) acid derivatives[10] confirms the assigned structure.

C_5 Acids. — Spectrum I in Fig. 3 was recorded for the major dicarboxylic acid from oxygen–alkali treatment of xylan. The peak at *m/e* 437 indicates a C_5 deoxyaldaric acid (mol. wt., 452). The M−44 peak is consistent with 2-(2-hydroxyethyl)-tartronic acid (*cf.* Fig. 1C). This structure is confirmed by the formation of *m/e* 335 ions, which are likely to decompose further by heterolysis to *m/e* 103 ions. A metastable peak demonstrates that the *m/e* 335 ions also decompose to *m/e* 305 ions. The same metastable transition was observed for the identical *m/e* 335 ions from 3-deoxy-2-*C*-(hydroxymethyl)tetronic acid[9].

Spectrum II in Fig. 3 was obtained from one of the dicarboxylic acids isolated after alkali treatment of hydrocellulose[8]. The *m/e* 437 (M−15) peak suggests an isomeric C_5 deoxyaldaric acid, and the M−30 peak indicates 2-deoxy-3-*C*-(hydroxymethyl)tetraric acid (*cf.* Fig. 1B). The *m/e* 335 and *m/e* 349 α-cleavage ions confirm this structure.

A comparison of the spectra of the two identified C_5 acids with that of the structurally related 3-deoxy-2-*C*-(hydroxymethyl)tetronic acid reveals anticipated fragmentation analogies. Inspection of the structures of the five other constitutionally isomeric branched-deoxyaldaric acids demonstrates that none is compatible with the spectra discussed. Spectra of 2- and 3-deoxypentaric acid derivatives have been published[10].

C_6 Acids. — Spectrum III in Fig. 3 was recorded for the main dicarboxylic acid obtained on oxygen–alkali treatment of cotton cellulose[6]. The peak at *m/e* 539 indicates a C_6 deoxyaldaric acid (mol. wt., 554), and the M−44 peak provides strong evidence for a tartronic acid structure with a CH_2R branch (Fig. 1C). The *m/e* 205 peak should correspond to a vicinal diol end-group and indicates 2-(2,3-dihydroxypropyl)tartronic acid. The *m/e* 451 and *m/e* 437 α-cleavage ions decompose by elimination of Me_3SiOH (90 mass units), and the presence of the corresponding peaks confirms the structure.

The last spectrum (IV) in Fig. 3 corresponds to a major dicarboxylic acid formed by alkali treatment of pectic acid[8]. The peak at *m/e* 539 (M−15) clearly suggests an isomeric C_6 deoxyaldaric acid. The small but significant M−30 peak would be expected only from a 3-deoxy-2-*C*-(hydroxymethyl)aldaric acid (Fig. 1B). Confirmatory peaks from α-cleavage ions (*m/e* 437 and 451) of high mass are present. The formation of 3-deoxy-2-*C*-(hydroxymethyl)pentaric acids from pectic acid is also consistent with their previously observed formation from alginates[4].

For both C_6 acids discussed, fragmentation paths and peak intensities are analogous to those of 3-deoxy-2-*C*-(hydroxymethyl)pentonic acids[9]. The differences in the relative abundance of the α-cleavage ions in spectra III and IV reflect the anticipated influence of a carbonyl group adjacent to the site of charge. The previously studied spectra of 2- and 3-deoxyhexaric acids are rather different[10].

Another acid obtained from the pectic material gave rise to a spectrum very similar to IV, and is therefore the diastereomeric acid. Since one of the products from reduction with potassium borohydride after lactonization was identified as 3-deoxy-2-*C*-hydroxymethyl-*threo*-pentonic acid, this acid is the *threo* isomer, and the first-discussed acid is the *erythro* isomer.

G.l.c. characteristics. — Retention data for the acids identified are given in Table I. The relationships between structure and retention are similar to those discussed for aldonic and deoxyaldonic acids[5] and unbranched aldaric and deoxyaldaric acids[7]. The analytical conditions were the same as in these previous studies, and the retention data are directly comparable. The retention of the deoxyaldaric acids relative to the less polar Me_3Si derivative of D-glucitol increases with increasing polarity of the stationary phase. An increased number of carbon atoms is reflected in a longer retention time. As with the corresponding deoxyaldonic acids, the branched C_4 and C_5 acids are eluted before the corresponding unbranched acids on each of the four stationary phases. The C_4 acid is eluted even before tartronic acid. The substituted tartronic acids, compared with the isomeric acids, are less strongly retained on QF-1 than on the other phases, and all the branched acids can be well separated on this phase. The favourable g.l.c. separation characteristics of the Me_3Si derivatives of hydroxy dicarboxylic acids is an additional advantage in the application of g.l.c.–m.s. to these acids.

TABLE I

G.L.C. DATA[a] FOR Me_3Si DERIVATIVES OF BRANCHED DEOXYALDARIC ACIDS: RELATIVE RETENTIONS[b]

	OV-1 160°	*OV-17* 160°	*QF-1* 120°	*XE-60* 120°
C_4-Acids				
2-Methyltartronic	0.058	0.101	0.110	0.100
C_5-Acids				
2-(2-Hydroxyethyl)tartronic	0.250	0.444	0.491	0.513
2-Deoxy-3-*C*-(hydroxymethyl)tetraric	0.271	0.466	0.582	0.562
C_6-Acids				
2-(2,3-Dihydroxypropyl)tartronic	0.767	1.278	1.368	1.538
3-Deoxy-2-*C*-hydroxymethyl-*erythro*-pentaric	0.816	1.357	1.940	1.936
3-Deoxy-2-*C*-hydroxymethyl-*threo*-pentaric	0.737	1.179	1.504	1.503

[a]Experimental data as in Ref. 5. [b]Adjusted retention times relative to those of the Me_3Si derivative of D-glucitol (12.0 min for OV-1; 6.1 min for OV-17; 17.3 min for QF-1; 15.0 min for XE-60).

DISCUSSION

3-Deoxy-2-C-(hydroxymethyl)aldaric acids. — The reaction sequence (Fig. 4A) leading to the formation of 3-deoxy-2-*C*-(hydroxymethyl)aldonic acids from 4-*O*-substituted reducing sugars is well established[1–3]. The same reactions with formation

of the corresponding aldaric acids are expected for penturonic ($R^1 = COOH$) and hexuronic [$R^1 = CH(OH)COOH$] acids. Using chemical evidence, Whistler and Richards demonstrated the formation of the C_6 acids from alginates and concluded that the same acids were formed from 4-*O*-methylglucuronic acid by the indicated reaction pathway[12]. Investigations concurrent with the present study confirm that the 3-deoxy-2-*C*-(hydroxymethyl)pentaric acids are major products from oxygen-free alkaline treatment of (1→4)-linked glycuronans and 4-*O*-alkylsubstituted hexuronic acids[8].

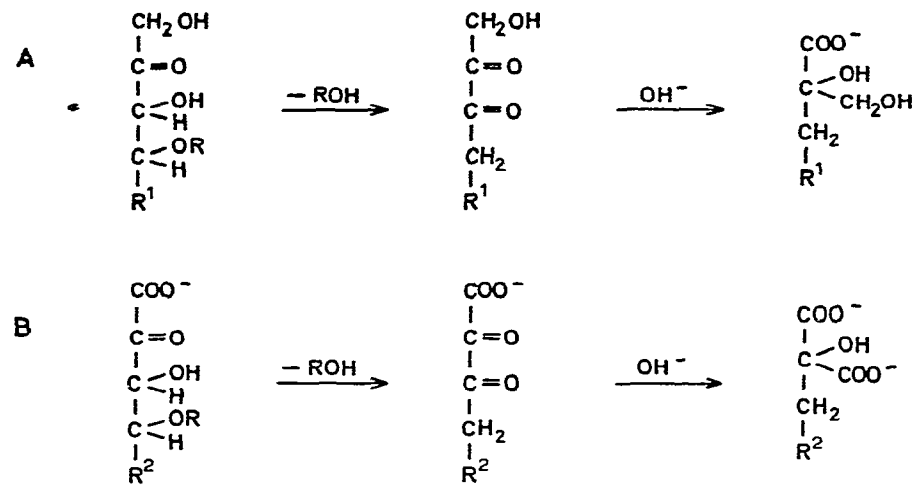


Fig. 4. Reaction schemes for the formation of 3-deoxy-2-*C*-(hydroxymethyl)aldaric acids (A) and 2-substituted tartronic acids (B).

Substituted tartronic acids. — The formation of substituted tartronic acids can be envisaged from 4-*O*-substituted 2-ulosonic acids (Fig. 4B) by analogy with the preceding reaction sequence. The β-elimination of the OR group is followed by a benzilic acid rearrangement of the resulting dicarbonyl intermediate. Hydrated forms of the dicarbonyl intermediate may be involved in the benzilic acid rearrangement[13] and make its course difficult to predict. Migrations of alkyl groups in the rearrangement are well-known, whereas evidence for the migration of carboxylate groups is scarce. However, the formation of 2-methyltartronic acid from the ethyl ester of 2,3-diketobutanoic acid has been investigated[14]. It was shown by ^{14}C-labelling that the methyl group does not migrate, but it was not proved that saponification precedes rearrangement.

The studies of oxygen–alkali treatments, which initiated this investigation, demonstrated that 2-(2-hydroxyethyl)tartronic acid is a major product from xylan[8] and that 2-(2,3-dihydroxypropyl)tartronic acid is a prominent product from cellulose[6] as well as cellobiose[8]. In these oxygen-alkali reactions, another postulated precursor to the acids is a 4-*O*-substituted and enolized ulosono-1,5-lactone, related in structure to the ascorbic acids.

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