Preparation of Methyl cis-9,cis-12,cis-15-Octadecatrienoate-15,16-d$_2$ and Methyl cis-9,cis-12,cis-15-Octadecatrienoate-6,6,7,7-d$_4$

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Methyl cis-9,cis-12,cis-15-octadecatrienoate-15,16-d$_2$ was obtained from Wittig coupling of methyl 12-oxo-cis-9-dodecenate, 18, and 3,4-dideutero-cis-3-hexenyltriphenylphosphonium bromide, 16. Compound 18 was obtained by periodic acid oxidation of methyl 12,13-dihydroxy-cis-9-octadecenoate, 17, obtained from Vernonia oil. Compound 18 also was synthesized from methyl oleate as the starting material. The deuterated fragment, 16, was prepared from 3-hexynol and using Lindlar's catalyst and deuterium gas to introduce the deuterium atoms.

Methyl cis-9,cis-12,cis-15-octadecatrienoate-6,6,7,7-d$_4$ was prepared by Wittig coupling of 3,6-nonadienyltriphenylphosphonium iodide, 5, with methyl 9-oxononanoate-6,6,7,7-d$_4$, 11. Deuterium atoms were introduced during the synthesis of 11 from 3-butylnol and 5-bromopentanoic acid with deuterium gas in the presence of [Ph$_3$P]$_2$RhCl. For the preparation of 5, the 3,6-nonadiynol intermediate was reduced to 3,6-nonadienol with P-2 Nickel and hydrogen.

The final products were separated from isomers formed during the synthetic sequences by silver resin chromatography.


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**Scheme 1**

DHP, Dihydropyran; THP, tetrahydropyran; p-TSA, p-toluene-sulfonic acid; THF, tetrahydrofuran.

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For our study of the metabolism in humans of fatty acids formed during the hydrogenation of soybean oil (1,2), we have synthesized various deuterated monoenoic (3), dienoic (4) and trienoic acids (5). In all cases, we have been able to avoid putting the deuterium atoms on the double-bonded carbon atoms or on the carbon atoms immediately adjacent to them. As the number of double bonds in the molecule is increased, it becomes more and more difficult to locate the deuterium atoms on carbons that are not joined by a double bond or are immediately adjacent to them. We now have synthesized methyl linolenate containing four deuterium atoms in the 6,6,7,7 positions and methyl linolenate containing two deuterium atoms on the #15 and #16 double-bonded carbon atoms for use in a study to ascertain if there is a “deuterium isotope effect” in the metabolic processes of humans.

**DISCUSSION**

Methyl linolenate-6,6,7,7-d$_4$, 12, was obtained by a Wittig coupling of two fragments that were prepared by acetylenic coupling (Scheme 1). For the preparation of 3,6-nonadienyltriphenylphosphonium iodide, 5, 2-pentynol was converted to 1-bromo-2-pentyne, 1, with triphenyl-
phosphine dibromide. Compound 1 then was coupled in a Grignard reaction with 3-butynol to give 3,6-nonadiynol, 2, which was reduced with P-2 Nickel (6) and hydrogen to 3,6-nonadienol, 3. (Lindlar's catalyst is reported [7] to give a mixture of isomeric products with diynes. By contrast, P-2 Nickel reduction goes rapidly to the diene stage in good yield.) Reaction of 3 with triphenylphosphine dibromide gave 1-bromo-3,6-nonadiene, 4, which was converted to the iodide with sodium iodide and to the phosphonium salt, 5, by reaction with triphenylphosphine in acetonitrile.

For the aldehyde ester required, methyl 9-oxononanoate-6,6,7,7-d4, 11, 3-butynol and 3-bromopentanoic acid were coupled by lithium amide in liquid ammonia to give 6, 9-hydroxy-6-nonynoic acid. (The same reaction with methyl 5-bromopentanoate gives a complex mixture of products.) To prevent deuterium-hydrogen exchange during the catalytic deuteration, 6 was esterified to 7 and the hydroxy group was protected as the tetrahydropyranyl ether, 8. Deuteration of 8 with deuterium gas in the presence of [tris(triphenylphosphine)chlororhodium gave methyl 9-(2-tetrahydropyranyloxy)nonanoate-6,6,7,7-d4, 9. The tetrahydropyranyl group was removed with methanol and p-toluenesulfonic acid to give 10, which was oxidized to 11 with pyridinium chlorochromate (8).

Wittig reaction between 5 and 11 with butyl lithium in tetrahydrofuran gave 12. The all-cis isomer was separated from the small amounts of the other isomers generated during the reaction sequence by silver resin chromatography (9). Methyl linolenate-15,16-d2, 19, was obtained by Wittig coupling (Scheme 2) of 3,4-dideutero-3-hexenyltriphenylphosphonium bromide, 16, and methyl 12-oxo-cis-9-dodecenoate, 18, with butyl lithium in tetrahydrofuran. The all-cis isomer was separated from the other isomers formed by silver resin chromatography (9). The synthesis of the phosphonium salt started with 3-hexynol, which was converted to the tetrahydropyranyl ether, 13, to prevent hydrogen-deuterium exchange in the subsequent deuteration reaction with deuterium gas and Lindlar's catalyst. The dideuterated tetrahydropyranyl ether, 14, was converted to the bromide, 15, with triphenylphosphine dibromide and to the phosphonium salt, 16, with triphenylphosphine in acetonitrile.

The unsaturated aldehyde ester, 18, was obtained by periodic acid oxidation, below 6 C (10), of methyl threo-12,13-dihydroxy-cis-9-octadecenoate, 17. This dihydroxy ester was obtained from Vernonia oil. The mixture of acids obtained from acetylation and saponification of Vernonia oil contained about 67% dihydroxyoleic acid. This was increased to 84% by distribution between aqueous acetonitrile and petroleum ether and to 96.8% by recrystallization from ethyl ether-petroleum ether.

Compound 18 is a very useful intermediate and was used in our synthesis of mono-, di- and trienoic fatty esters containing a 12,13 double bond (11). Since Vernonia oil is not commercially available and was available to us only in limited amounts, we devised a synthesis (Scheme 3) of this material from methyl oleate, which is readily available from the esterification of Palmolyn 100, a commercial source of oleic acid. Palmolyn 100 methyl esters (about 94% methyl oleate), 20, was ozonized, and the ozonide was reduced with zinc and acetic acid to yield 21, methyl 9-oxononanoate (methyl azelaidaldehydate). This compound was coupled (12) in a Wittig reaction with [2-(1,3-dioxan-2-y)ethyl]triphenylphosphonium bromide and butyl lithium in tetrahydrofuran to give the cis (75%) and trans (25%) isomers of methyl 11-dioxanyl-9-undecenoate, 22. The geometric isomers were separated on a 100% Ag/Na (9) column, and the cis isomer was transacetalized with methanol and p-toluenesulfonic acid (5) to methyl 12,12-dimethoxy-cis-9-dodecenoate, 23. This compound gave the same 13C NMR spectrum as the methyl 12,12-dimethoxy-cis-9-dodecenoate obtained by the periodic acid oxidation in methanol of methyl threo-12,13-dihydroxy-cis-9-octadecenoate obtained from Vernonia Seed Oil

Vernonia Seed Oil

1. Hydrolysis
2. Purification
3. DHP, Dihydropyran; THP, tetrahydropyranyl; p-TSA, p-toluene-sulfonic acid; THF, tetrahydrofuran.
Vernonia oil (10). Hydrolysis of the acetal ester yields the \( \beta, \gamma \)-unsaturated aldehyde ester contaminated with small amounts of the \( \alpha, \beta \)-isomer, the quantity of which depends on the reagents and conditions used for the hydrolysis.

If the Wittig reaction between 21 and the dioxanyl-ethylphosphonium salt is carried out under thermodynamic control (4), the reaction mixture will contain a larger percentage of the trans isomer of 22. This can be separated and transacetalized to methyl 12,12-dimethoxy-trans-9-dodecenolate, which can be used to make other isomers.

**EXPERIMENTAL**

**Reagents.** [2-(1,3-Dioxan-2-yl)ethyl]triphenylphosphonium bromide, butyl lithium, triphenylphosphine, dihydropyran, pyridinium chlorochromate, ethyl magnesium bromide, sodium borohydride, 5-bromopentanoic acid, phosphine)chlororhodium was obtained from Strem Chemicals (Newburyport, MA) and Palmolyn 100 from Hercules, Inc. (Wilmington, DE).

**Procedures.** A 50 m X 0.25 mm CPS-2 capillary column (Quadrex Corp., New Haven, CT) was used for analyzing binary mixtures of geometric isomers. For other analyses, a 6 ft X 4 mm column packed with 3% EGSSX on 100/120 GasChrom Q or a 6 ft X 4 mm column packed with 3% OV101 on 80/100 Supelcoport was employed.


H. RAKOFF
in 95% ethanol (150 ml). The borohydride solution (19 ml, 20 mmol) was added via syringe. There was a rapid evolution of gas, and the mixture turned black. Ethylenediamine (2 ml) was added, and the system was evacuated and flushed several times with H₂. Then, 3,6-nonadiynol (22.64 g, 166 mmol) in 95% ethanol (25 ml) was added, and stirring was started. When absorption of H₂ had almost ceased, the reaction mixture was filtered through a pad of Celite and Darco 6-60 in a Buchner funnel. The pad then was washed with 95% ethanol (2 X 250 ml). The combined ethanol extracts, 2 which had a permanganate color, were concentrated on the rotary evaporator to 65 ml. Water (100 ml) was added, and the mixture was extracted with ethyl ether (3 X 40 ml). The combined ether extracts were washed with saturated NaCl solution (2 X 40 ml) and dried over Na₂SO₄. Distillation gave compound 3 (19.08 g, 81.9% yield), bp 92-98°C @ 4.5 torr. ¹³C NMR in CDCl₃ (C#, ppm): 1, 61.95; 2, 30.67; 3, 131.94; 4, 126.17; 5, 25.51; 6, 125.32; 7, 130.93; 8, 20.42; 9, 14.06. Preparation: 1-bromo-6,6-nonadiene, 4. A manner similar to the conversion of 2-benzyl to 1-bromo-2-pentyne, 1, 3,6-nonadienyl, 3, (19 g, 136 mmol) was treated with triphenylphosphine dibromide to give compound 4 (23.4 g, 85% yield), bp 82-89°C @ 3.75 torr. ¹³C NMR in CDCl₃ (C#, ppm): 1, 32.36; 2, 30.83; 3, 131.22; 4, 126.51; 5, 25.69; 6, 126.06; 7, 132.27; 8, 20.57; 9, 14.19.

Preparation: 3,6-nonadienyltriphenylphosphonium iodide, 5. A solution of 4 (23.4 g, 115 mmol) in acetone (50 ml) was added to a solution of NaI (21.6 g, 144 mmol) in acetone (100 ml). The mixture was heated at the reflux temperature for 1.5 hr; the white solid was removed by filtration; and the filtrate was concentrated on the rotary evaporator to an orange liquid and yellow solid (total weight 33.07 g). The liquid was decanted into a 500 ml round bottom flask, and the yellow solid was washed with acetonitrile, which was added to the flask. Triphenylphosphine (35 g, 134 mmol) was added together with the remainder of 200 ml of acetonitrile. Xylene (1 ml) was added as an internal standard, and the reaction mixture was heated at the reflux temperature by means of an oil bath. Samples were analyzed on a 3% OV101 column. After three hr, only 16.6% of the iodo compound remained; after 19 hr, only 1.5% remained. Solvent was removed by distillation under reduced pressure in the presence of a pellet of KOH to give compound 5 (67.8 g, 96% yield) bp 140-147°C @ 0.2 torr. ¹³C NMR in CDCl₃ (C#, ppm): 1, 173.5; 2, 33.4; 3, 23.9; 4, 28.2; 5, 18.3; 6, 80.3; 7, 77.2; 8, 15.7; 9, 66.0; OCH₃, 51.2. THP group: 2, 98.5; 3, 30.5; 4, 19.3; 5, 25.4; 6, 61.9.

Preparation: methyl 9-(2-tetrahydropyranyloxy)-6-nonynoate, 8. Methyl 9-hydroxy-6-nonynoate (7 g, 38 mmol) and p-toluenesulfonic acid (0.5 g) were dissolved in ethyl ether (10 ml) in a 100 ml round bottom flask equipped with a magnetic stirring bar and a reflux condenser. Dibhydroxypyrane (4 g, 48 mmol) in ethanol (10 ml) was added dropwise. Heat was evolved, and the reaction mixture darkened. After three hr, the reaction mixture was washed with saturated Na₂CO₃ solution (10 ml), dried and distilled under reduced pressure in the presence of a pellet of KOH to give compound 8 (6.74 g, 66% yield) bp 140-147°C @ 0.2 torr. ¹³C NMR in CDCl₃ (C#, ppm): 1, 173.5; 2, 33.4; 3, 23.9; 4, 28.2; 5, 18.3; 6, 80.3; 7, 77.2; 8, 15.7; 9, 66.0; OCH₃, 51.2. THP group: 2, 98.5; 3, 30.5; 4, 19.3; 5, 25.4; 6, 61.9.

Preparation: methyl 9-(2-tetrahydropyranyloxy)nonanoate-6,7,7-d₄, 9. Compound 8 (6.7 g, 25 mmol) in benzene (200 ml) was treated with deuterium gas at atmospheric pressure in the presence of tris(triphenylphosphine)chlororhodium (1 g) in the manner described previously (8). The benzene was removed on the rotary evaporator, and the residue was passed through a column (1.5 x 25 cm) of silica gel (15 g) with hexane to remove the catalyst. Removal of the solvent gave compound 9 (6.63 g, 96% yield). ¹³C NMR in CDCl₃ (C#, ppm): 1, 174.2; 2, 34.1; 3, 24.9; 4-5, 28.9-29.0; 6, 29.4; 7, 67.6; OCH₃, 51.3: THP group: 2, 98.8; 3, 30.8; 4, 19.7; 5, 25.4; 6, 62.3.

Preparation: methyl 9-hydroxynonanoate-6,7,7-d₄, 10. Compound 9 (6.63 g, 24 mmol) was stirred in methanol (150 ml) with p-toluene sulfonic acid (0.35 g) in a nitrogen atmosphere. Two hr later, solid Na₂CO₃ was added, and the mixture was concentrated to a thick liquid on the rotary evaporator. Saturated Na₂CO₃ solution (40 ml) was added, and the mixture was extracted into ethyl ether (2 X 25 ml) and dried over Na₂SO₃. The drying agent and solvent were removed and compound 10 (3.62 g, 78.7% yield) was obtained by distillation, bp 113–120°C @ 0.35 torr. ¹³C NMR in CDCl₃ (C#, ppm): 1, 174.2; 2, 34.0; 3, 24.8; 4-5, 28.8-28.9; 6, 32.4; 7, 62.7; OCH₃, 51.3.

Preparation: methyl 9-oxononanoate-6,7,7-d₄, 11. Pyridinium chlorochromate (11.54 g, 53 mmol) was slurried
in CH₂Cl₂ (100 ml, dried over Molecular Sieve 4 Å) in a 250 ml three-necked flask equipped with a mechanical stirrer, a thermometer and a CaCl₂ drying tube. Compound 10 (6.8 g, 35.4 mmol) dissolved in CH₂Cl₂ (20 ml) was added to the orange slurry, which darkened rapidly and deposited a tarry material on the walls of the flask. The temperature was maintained at 20 ± 2°C by intermittent use of an ice bath. Two hr later, ethyl ether (100 ml) was stirred into the mixture and then decanted. This was repeated three times with 25 ml portions of ether. The combined decantates were passed through Florisil (20 g) in a column (1.5 X 25 cm), and the column was flushed with ether (50 ml). The solvents were removed on a rotary evaporator to give a green liquid (6.67 g), that was distilled to give compound 11 (4.47 g, 66.7% yield), bp 96-102°C @ 0.35 torr. 13C NMR in CDCl₃ (C# ppm): 1, 174.0; 2, 33.9; 3, 24.8; 4-5, 28.6-28.8; 8, 43.5; 9, 202.6; OCH₃, 51.3.

Preparation: methyl cis-9-cis-12-cis-15-octadecatrien-ate-6,7,7-d₄, 12. A slurry of 5 (13.5 g, 26.4 mmol) in tetrahydrofuran (100 ml) was prepared in a 250 ml three-necked flask equipped with a mechanical stirrer, a low temperature thermometer, a dropping funnel and a CaCl₂ drying tube. A stream of N₂ was passed through the flask and it was cooled in an ice-salt bath to about 1°C. Butyl lithium in hexane (2.42 M, 15 ml, 36 mmol) was added over six hr. The reaction mixture developed a pale pink-amber color. While a stream of N₂ was passed through the mixture and then decanted. The apparatus was evacuated and flushed three times with D₂. Then, Lindlar’s catalyst (1 g) and quinoline (0.8 ml) were added, and a pressure of one atmosphere of D₂ was maintained as the reaction mixture was stirred rapidly. After absorption of D₂ had ceased, the reaction mixture was filtered through a pad of Celite to remove the catalyst. Ethyl acetate was removed by distillation at atmospheric pressure and compound 14 (41.91 g, 91% yield) was obtained by distillation, bp 63-68°C @ 0.4 torr. Mass spectral analysis indicated better than 99% d₄.

Preparation: 1-bromo-3-hexene-3,4-d₂, 15. In a manner similar to the conversion of 2-pentynol to 1-bromo-2-pentynyl, 1, compound 14 was converted to compound 15 in 72% yield, bp 120-140°C. Compound 15 tends to codistill with the solvents employed.

Preparation: 3,4-dideutero-cis-3-hexenyltriphenylphosphonium bromide, 16. Triphenylphosphine (28.8 g, 110 mmol), compound 15 (17.8 g, 108 mmol) and toluene (1 ml) were mixed in acetonitrile (110 ml) in a 250 ml round bottom flask equipped with a magnetic stirring bar and a reflux condenser. The mixture was heated with stirring at the reflux temperature, and the ratio of bromohexene to toluene was followed by GLC on an OV101 column. After 30 hr, only about 9% of bromohexene remained. Acetonitrile (45 ml) was removed on the rotary evaporator, and the residue was triturated several times with fresh portions of ethyl ether. The white solid that formed was dried in a vacuum desiccator to give compound 16 (39.8 g, 86.4% yield), mp 164-167°C. 13C NMR in CDCl₃ (C#, ppm); 1, 23.07; 2, 20.15; 5, 20.03; 6, 13.7; aromatic ring: 1, 118.03; 2, 133.50; 3, 130.37; 4, 134.95.

Preparation: methyl 12-oxo-cis-9-dodecenoate, 18. Methyl 12,13-dihydroxy-cis-9-octadecenoate (50 g, 152 mmol) was dissolved in tetrahydrofuran (150 ml) in a 500 ml Erlenmeyer flask equipped with a magnetic stirring bar and a low-temperature thermometer and immersed in an ice-salt bath. Paraperiodic acid, H₂IO₆ (43.5 g, 190 mmol) was dissolved in H₂O (87 ml) and cooled in an ice bath. The periodic acid solution was added in portions over 40 min so that the temperature did not rise above 4°C. The reaction mixture was diluted with H₂O (150 ml), the organic layer was separated, and the aqueous layer was extracted with hexane (2 X 50 ml). The combined organic layers were washed with H₂O (1 X 25 ml) and dried over Na₂SO₄ and Drierite. After removal of the drying agents and solvent, the residue was freed of any other volatiles (especially hexanal) by use of a vacuum pump at room temperature. The residue was then distilled through a falling film molecular still (0.05 torr, refluxing xylene) to give compound 18 (27.36 g, 79% yield).

Preparation: methyl cis-9-cis-12-cis-15-octadecatrien-ate-15,16-d₄, 19. Compound 16 (39.03 g, 91.4 mmol) was slurried in tetrahydrofuran (200 ml) in a 500 ml three-necked flask equipped with a mechanical stirrer, a low-temperature thermometer, a dropping funnel and a CaCl₂ drying tube. A stream of N₂ was maintained through the apparatus as it was cooled in an ice-salt bath. Butyl lithium in hexane (2.5 M, 40 ml, 100 mmol) was added in portions with the temperature varying between 0 and 18°C. Then compound 18 (27.25 g, 120 mmol) in tetrahydrofuran (10 ml) was added over 12 min to the blood
SYNTHESIS OF DEUTERIUM LABELED METHYL LINOLENATES

red reaction mixture. The maximum temperature was 7°C, and the reaction mixture formed a pale-tan slurry. The ice bath was removed, and about one hr later the reaction mixture was shaken with saturated NaCl solution (100 ml). The organic layer was dried over Na2SO4 and Drierite. After filtration, the solvent was removed on the rotary evaporator, and the residue (71.71 g) was placed on a column (3 X 50 cm) containing silica gel (100 g) in hexane. Elution with hexane yielded a mixture of isomers (19.81 g, 73.7% yield). This mixture was separated on a 5 X 82 cm column packed with Amberlyst XN1010 (40/80 mesh) 100% Ag/H resin (9) with the eluant being acetone (300 ml) in methanol (3.7 l), to give the all-cis isomer (7.69 g, 28.4% yield based on the phosphonium salt used). Mass spectral analysis: 0.41% d9, 0.29% d11, 99.26% d12.

13C NMR in CDCl3 (Cδ, ppm): 1, 174.1; 2, 34.0; 3, 24.8; 4-7, 29.0-29.4; 8, 27.1; 9, 150.1; 10, 127.6; 11, 25.3-25.5; 12, 128.1; 13, 128.1; 14, 25.3-25.5; 17, 20.3; 18, 14.1.

Preparation: methyl 9-oxononanolate, 21. Compound 21 was prepared in 64% yield essentially as previously described (14) but with the use of Sudan Red (0.01 g in 10 g of cyclohexane) as an indicator of the completion of the ozonolysis and Palmolyn 100 methyl esters as the source of methyl oleate.

Preparation: methyl 11-dioxanyl-9-undecenoates, 22. Compound 22, bp 140-152°C @ 0.2 torr, was obtained in 78% yield by the method previously described (5,12) for the synthesis of another dioxanyl ester. Mass spectroscopy showed a parent peak at 284 mlz. and a large peak at 87 mlz corresponding to the dioxanyl group. 13C NMR chemical shifts were consistent with the structure assigned. The cis and trans isomers of 22 (73.4% cis) were separated on a 3.5 X 30 cm column of 100% Ag/Na (9) Amberlyst XN1010 ion exchange resin (200-270 mesh). From a sample of 1.84 g, there was obtained 1.19 g (88% of theory) of the cis isomer.

Preparation: methyl 12,12-dimethoxy-cis-9-dodecenolate, 23. Compound 23, bp 132-140°C @ 0.25 torr, was obtained in 62% yield from 22 by a method described (5,12). The 13C NMR spectrum is identical to that of the dimethyl-acetel ester prepared by periodic acid oxidation in methanol (10) of methyl 12,13-dihydroxy-cis-9-octadecenoate. During GC/MS analysis, the dimethyl acetal apparently loses CH3OH to give methyl 12-methoxy-9,11-dodecadioenate, which gives a parent peak at 240 m/z and an M-31 peak at 209 m/z. The base peak is at 97 m/z corresponding to CH3OCH2(CH=CH)2.

Purification of 12,13-dihydroxy-cis-9-octadecenoic acid. (1) By distribution between aqueous acetonitrile and petroleum ether. The mixture of acids obtained from the acetylsis and saponification of Vernonia anthelmintica seed oil (15) was analyzed by GLC on 3% EGSSX after the acid group had been converted to the methyl ester and the hydroxy groups had been converted to a dioinoane with aceton and BF3CH2OH. This acid mixture (301 g) showed 67% dihydroxyoleic acid. It was suspended in a mixture of acetonitrile (700 ml) and H2O (70 ml) and extracted with petroleum ether (3 X 250 ml). Removal of the CH3CN-H2O gave a product (204.3 g) that analysis showed to contain 84% dihydroxyoleic acid.

(2) By crystallization from ethyl ether-petroleum ether. A sample (230 g) of Vernonia acids containing 84% dihydroxyoleic acid was dissolved with stirring and washed with petroleum ether to remove a small amount of containing dark oil. The solid was pulverized and air-dried to give a product (61.26 g) analysis of which showed 96.6% dihydroxyoleic acid, 1.57% linoleic acid, 0.38% oleic acid, 0.36% palmitic acid and 1.04% of a material eluting after dihydroxyoleic acid. Mp 49-50°C.

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REFERENCES


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