α-METHYLENE-γ-LACTONE FUSED TO STEROIDAL RING D AS POTENTIAL ANTITUMOR AGENT

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ABSTRACT

Steroidal α-methylene-γ-lactone 1a has been synthesised from 3β-hydroxy-5-androsten-17-one 2 and showed to be active against HeLa cells.

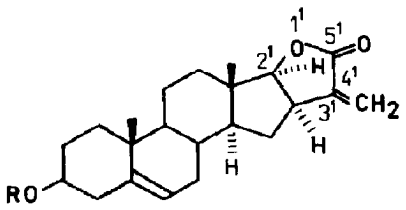
Cytotoxic activity inherent in several sesquiterpenes such as elephantopin, vernolepin, and helenalin has been attributed to the presence in their structure of an α-methylene-γ-lactone moiety, and explaned by a rapid and essentially irreversible reaction of this functional group with biological nucleophiles (1). The activity of these plant derived compounds is overshadowed by their high toxicity and poor therapeutic indices, which preclude their clinical use (2). In the search for drugs for cancer chemotherapy which would exert a more selective action, the α-methylene-γ-lactone grouping has been attached to the steroid nucleus at the positions 3 and 6 in spiro-fashion (3). Recently other steroidal carriers of an α-methylene-lactone function have been synthesised and found to possess antitumor properties (4,5,6).

The aim of this work was to synthesise and examine the cancerostatic activity of the α-methylene-γ-lactone deriva-

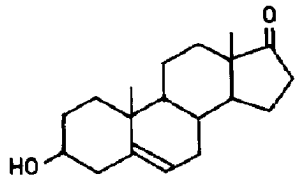
tive 1 in which the structure of rings A-D is typical of natural steroids, and the lactone ring is condensed to ring D at C_{16} and C_{17}.

Ketone 2 was transformed by Kurath and Cole's method (7) via the hydroxy keto acid 3 into the dihydroxy acid 4a, diacetoxy methyl ester 4c of which gave upon partial hydrogenation in acetic acid, over a platinum catalyst, the diacetoxy ester 5. Treatment of this product with aqueous potassium hydroxide followed by acidification resulted in hydrolysis of the ester groups and closure of the 5-membered lactone ring. The lactone 6a showed physical and spectroscopic properties in agreement with those described (7).

In order to introduce a methylene group at the lactone ring of compound 6a, the hydroxy group was protected as tetrahydropyranyl (THP) ether, and the derivative 6b was treated in tetrahydrofuran (THF) with lithium diisopropylamide (LDA) followed by methyl iodide. The alkylation product 6c, isolated in 94% yield, showed in its ^1H NMR spectrum a doublet at δ 1.28 ppm, integrating for 3H, J= 7 Hz, which was assigned to the methyl group at the position 4^1. It was assumed that methylation of the lactone 6a proceeds from the exo side of the cis-fused 5-membered rings which would result in a product with an α-oriented methyl group. The methyl lactone 6c was treated sequentially with LDA and phenylselenyl bromide. A crystalline selenide 6d was obtained in 87% yield; the structure of the product 6d



1a R=H
1b R=THP



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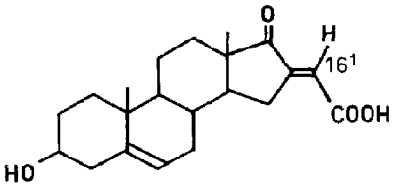

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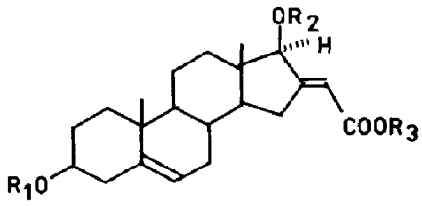
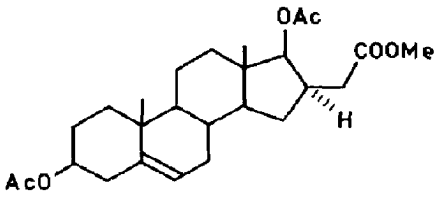


4a $R_1=R_2=R_3=H$
4b $R_1=R_2=H$, $R_3=Me$
4c $R_1=R_2=Ac$, $R_3=Me$



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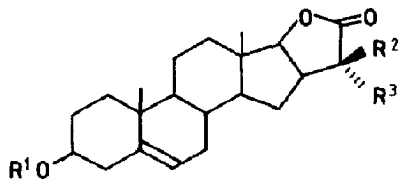
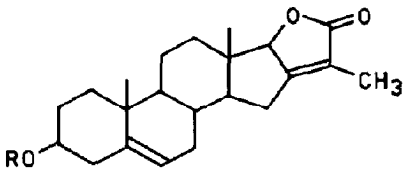


6a $R^1=R^2=R^3=H$
6b R^1=THP, $R^2=R^3=H$
6c R^1=THP, $R^2=H$, $R^3=Me$
6d R^1=THP, $R^2=Me$, $R^3=SePh$
6e R^1=THP, $R^2=H$, $R^3=SePh$
6f R^1=THP, $R^2=SePh$, $R^3=Me$



7a R=THP
7b R=H

was substantiated by analytical and spectroscopic data, the most indicative being a singlet at δ 1.55 ppm integrating for 3H in its ^{1}H NMR spectrum, which corresponds to the 4^1-methyl group. The selenide 6d was oxidized at 0°C with hydrogen peroxide in THF containing acetic acid whereupon the formation of a selenoxide and the elimination of phenylselenic acid took place. The product of this reaction (91% yield) had an IR spectrum of ν_{max} 1765 and 1695 cm^{-1} corresponding to an α,β-unsaturated lactone; however, in its ^{1}H NMR spectrum there were no signals for the methylene protons. Instead, a sharp singlet for 3H at δ 1.87 ppm appeared, suggesting that the α-methyl lactone 7a was formed. The structure of compound 7a was confirmed by other spectral and analytical data, and by hydrolysis to the alcohol 7b, which showed analytical and spectral properties in accord with those expected.

The formation of the lactone 7a provides evidence that the phenylselenyl group and the proton on C_{16} are located at the same face of the molecule, because cis - elimination of phenylselenic acid under the conditions of the reaction is well documented. Therefore, an electrophilic attack of phenylselenyl bromide on the lithium enolate generated from the methyl lactone 6c proceeds from the α-side (exo) of the molecule. Inspection of a molecular model of compound 6d reveals that the methyl and methine protons are equally available for the selenoxide

moiety, and that there is no distinct difference in stability between the two possible isomers which would result in selenoxide elimination. However, exclusive formation of the endo product 7a is in compliance with the dipole - dipole interaction of the lactone carbonyl and selenoxide group as postulated by Trost et al.(8) for the analogous fragmentation of α-methyl-α-(methylsulfinyl)-γ-butyrolactones.

At this point it become clear that the synthetic route to the methylene lactone 1 should lead through the intermediate 6f in which the phenylselenyl group and the 16α hydrogen are in the trans configuration. Accordingly, the lactone 6a was treated with LDA and phenylselenyl bromide to give the phenylselenyl derivative 6e in 91% yield. In the ^{1}H NMR spectrum of compound 6e, a doublet at δ 3.69 ppm, J=7 Hz, integrating for 1H appeared. This signal was ascribed to the proton at the carbon bearing the phenylselenyl group. The phenylselenyl lactone 6e was then methylated with LDA and methyl iodide. The product 6f (87% yield) was more polar (R_f=0.51, benzene - ethyl acetate 9:1) in thin layer chromatography than its previously obtained epimer 6d (R_f=0.57). Oxidation of the phenylselenide 6f with hydrogen peroxide in THF at 0°C led to a single product in 93% yield. In its ^{1}H NMR spectrum, two doublets appeared at δ 6.18 and 5.56 ppm, J=3 Hz, each corresponding to 1H. These signals together with other analytical and

spectral data indicate that the α-methylene-γ-lactone 1b was formed as expected. The mild acid hydrolysis of the acetal linkage in the THP-derivative 1b afforded the target compound 1a.

The compound 1a was evaluated *in vitro* for cytotoxic activity against HeLa (human carcinoma cervix uteri) cells. The assays were conducted following the Cancer Chemotherapy National Service Centre (CCNSC) protocol for KB cells (9) and based on inhibition of the growth of HeLa cells in culture. The ED_{50} value for the compound 1a is estimated for 5.7 μg/mL. Further biological evaluation of this compound is in progress.

EXPERIMENTAL PART

Melting points were determined on a Kofler hot-stage apparatus. The spectra were recorded with the following instruments: IR-Beckman 4240 or Unicam SP200; UV-Beckman MIV; NMR-Jeol JNM-4H-100 (unless otherwise stated in $CDCl_3$ solutions); mass spectra-LKB2091 (at 15 eV ionization potential). Chemical shifts are reported in δ units, parts per million (ppm) downfield from tetramethylsilane. Column chromatography was performed on kieselgel 60, and TLC on silica gel G, Merck. Organic solutions were dried over anhydrous Na_2SO_4, and solvents were removed *in vacuo* on a rotary evaporator. Yields refer to products homogenous on TLC. Experiments are numbered to facilitate reference to description of procedures which are used repeatedly. Microanalyses were performed in our analytical laboratory.

1. 3β,17β-Dihydroxy-5-androsten-16-ylidenacetic acid methyl ester 4b

3β,17β-Dihydroxy-5-androsten-16-ylidenacetic acid 4a (7)(6.4 g) in ether (300 mL) was treated at 0°C with an excess of diazomethane in ether. The ester 4b (6.51 g) was isolated in usual way, mp 249-251°C (MeOH), λ max (EtOH) 226 nm, ε =13200, ν max $(KBr)_1$ 3510 (OH), 1695 (C=O), 1665 (C=C), and 1225 (C-O-C) cm^{-1}, NMR (pyridine) 0.87 and 1.07 (singlets, angular CH_3), 3.70 (3H, s, $COOCH_3$), 3.80 (1H, m, C_3-H), 4.26 (1H, s, C_{17}-H), 6.49 (1H, m, C_{16}-H). Anal.Calcd.

for $C_{22}H_{32}O_4$: C, 73.33; H, 8.89%. Found: C, 73.34; H, 9.17%

2. 3β,17β-Diacetoxy-5-androsten-16-ylidenacetic acid methyl ester 4c

The diol 4b (6.25 g) was treated overnight at room temperature (RT) with acetic anhydride (50 mL) and pyridine (100 mL). The product was isolated with ether to give compound 4c (7.31 g, 93%) mp 181-182°C (MeOH), λ max (EtOH) 221 nm, ε =15700, ν max(KBr) 1730 (C=O), 1670 (C=C) cm^{-1}, NMR 0.74 and 1.04 (2s, angular CH_3), 2.02 (3H, s, C_3-$OCOCH_3$), 2.15 (3H, s, C_{17}-$OCOCH_3$), 3.70 (3H, s, $COOCH_3$), 4.59 (1H, m, C_3-H), 5.30, 1H, s, C_{17}-H), 5.39 (1H, m, C_6-H), 5.70 (1H, t, J=3 Hz, C_{16}-H). Anal. Calcd. for $C_{26}H_{36}O_6$: C, 70.23; H, 8.17%. Found: C, 70.26; H, 8.22%.

3. 3β,17β-Diacetoxy-5-androsten-16β-ylacetic acid methyl ester 5

A mixture of compound 4c (6.55 g), platinum oxide (200 mg), and acetic acid (50 mL) was shaken in a hydrogen atmosphere under atmospheric pressure until 375 mL of hydrogen was absorbed. The catalyst was filtered off, the filtrate was concentrated, and the product was isolated with chloroform. Compound 5 (5.83 g, 89%) was obtained, mp 132-135°C (MeOH), ν_{max} ($CHCl_3$) 1730 (C=O), 1250 (C-O-C)cm^{-1} NMR 0.80 and 1.03 (2s, angular CH_3), 2.02 and 2.03 (2s, $OCOCH_3$), 3.64 (3H, s, $COOCH_3$), 4.60 (1H, m, C_3-H), 4.81 (1H, d, J=10 Hz, C_{17}-H), 5.36 (1H, m, C_6-H). Anal. Calcd. for $C_{26}H_{38}O_6$: C, 69.96; H, 8.52%. Found: C, 70.08; H, 8.35%

4. 3β-Hydroxy-4^1,5^1,16α,17α-tetrahydro-5-androsteno[17, 16-b]furan-5^1-one 6a

A solution of compound 5 (5.6 g) in methanol (200 mL) containing water (20 mL) and KOH (5 g) was heated under reflux for 1.5 hrs, cooled, concentrated, and diluted with water. Then the mixture was acidified with 5% HCl and the product was isolated with chloroform to give the lactone 6a (3.87 g, 93%), mp 235-237°C (acetone). Described (7): mp 236-239°C.

5. 3β-Hydroxy-4^1,5^1,16α,17α-tetrahydro-5-androsteno[17, 16-b]furan-5^1-one 3-tetrahydropyranyl ether 6b

A mixture of the alcohol 6a (3.10 g, 9.4 mmol), dichloromethane (70 mL), dihydropyran (1.03 mL, 11.3 mmol), and p-toluenesulfonic acid (p-TSA)(150 mg) was stirred at RT 3 hrs, then washed with sat. aq. $NaHCO_3$ and the product was isolated in usual way. The THP-derivative 6b was obtained (3.49 g, 89%), mp 159-162°C (MeOH), ν max ($CHCl_3$) 1765 cm^{-1}, NMR 0.75 and 1.03 (2s, angular CH_3), 3.49 (2H, THP-H), 3.88 (1H, m, C_3-H), 4.37 (1H, d, J=9 Hz, C_{17}-H), 4.70 (1H, THP-H), 5.32 (1H, m, C_6-H). Anal. Calcd. for $C_{26}H_{38}O_4$: C, 75.36 H, 9.18%. Found: C, 75.29; H, 9.30%.

6. 4^1(R)3β-Hydroxy-4^1-methyl-4^1,5^1,16α,17α-tetrahydro--5-androsteno[17,16-b]furan-5^1-one 3-tetrahydropyranyl ether 6c

In an argon atmosphere, there was added to a stirred mixture of diisopropylamine (140 μL, 1.04 mmol) and THF (2 mL), at -5°C, n-butyllithium (0.65 mL of 1.6 M solution in hexane, 1.04 mmol). After 15 min the mixture was cooled to -78°C, and a solution of the lactone 6b (360 mg, 0.87 mmol) in THF (3 mL) was added dropwise over a period of 30 min. The mixture was stirred at -78°C for 30 min and treated with a mixture of methyl iodide (65 μL, 1.04 mmol), hexamethylphosphoric triamide (HMPT)(180 μL, 1.04 mmol), and THF (1 mL). Then the temperature was allowed to rise in 3 hrs to 22°C, and the reaction was quenched with 10% HCl. The product was isolated by means of benzene extraction and chromatography on a silica gel column (5 g) to give the lactone 6c (374 mg, 94%), mp 190-193°C (MeOH), ν max (KBr) 1765 (C=O) cm^{-1}, NMR 0.73 and 1.02 (2s, angular CH_3), 1.28 (3H, d, J=7 Hz, $C_4$1-CH_3), 3.50 (2H, THP-H), 3.89 (1H, m, C_3-H), 4.26 (1H, d, J=10 Hz, C_{17}-H), 4.70 (1H, THP-H), 5.34 (1H, m, C_6-H). Anal.Calcd. for $C_{27}H_{40}O_4$: C, 75.70; H, 9.35% Found: C, 75.75; H, 9.56%.

7. 4^1(R)3β-Hydroxy-4^1-methyl-4^1-selenophenyl-4^1,5^1,16α,17α-tetrahydro-5-androsteno[17,16-b]furan-5^1-one 3-tetrahydropyranyl ether 6d

The methyl lactone 6c was treated with phenylselenyl bromide in an analogous manner as the lactone 6b with methyl iodide in the Experiment 6. The reagents were used as follows: diisopropylamine (126 μL, 0.90 mmol) in THF (2 mL), n-butyllithium (0.56 mL of 1.6 M solution in hexane, 0.90 mmol); the lactone 6c (324 mg, 0.76 mmol) in THF (3 mL), phenylselenyl bromide (213 mg, 0.90 mmol) and HMPT (157 μL, 0.90 mmol) in THF (2 mL).

Chromatography of the crude product on a silica gel column (5 g, elution: hexane- ethyl acetate, 97:3) gave seleno derivative 6d (384 mg, 87%), mp 187-189° (MeOH), ν max (KBr) 3060 (arom.), 1765 (C=O) and 1580 (arom.) cm^{-1}, NMR 0.63 and 1.00 (2s, angular CH_3), 1.55 (3H, s, $C_4$1-CH_3), 3.53 (2H, m, THP-H), 3.88 (2H, d, J=10 Hz overlaping m, C_{17}-H and C_3-H), 4.72 (1H, THP-H), 5.31 (m, C_6-H), 7.2-7.4 and 7.55-7.75 (aromatic H). Anal. Calcd. for $C_{33}H_{44}O_4Se$: C, 67.92; H, 7.55%. Found: C, 67.84; H, 7.48%.

8. 3β-Hydroxy-4^1-methyl-5^1,16α-dihydro-5-androsteno[17,16-b]furan-5^1-one 3-tetrahydropyranyl ether 7a

To a vigorously stirred solution of the lactone 6d (283 mg, 0.49 mmol) in THF (2 mL), cooled to 0°C, acetic acid (0.1 mL) was added followed by 30% hydrogen peroxide (0.2 mL). After 15 min the reaction was quenched with sat. aq. $NaHCO_3$. The product was isolated with benzene and chromatogrphed on a silica gel column (2 g, elution: hexane -

ethyl acetate, 95:5) to give compound 7a (188 mg, 91%), mp 213-215°, λ max (EtOH) 222 nm, ε=11300, ν max(KBr) 1765 and 1695 (CO-C=C) cm^{-1}, NMR 0.53 and 1.02 (2s, angular CH$_3$), 1.87 (3H, s, C$_4$1-CH$_3$), 3.50 (2H, THP-H), 3.90 (1H, m, C$_3$-H), 4.53 (1H, s, C$_{17}$-H), 4.72 (1H, THP-H), 5.33 (1H, m, C$_6$-H). Anal. Calcd. for $C_{27}H_{38}O_4$: C, 76.06; H, 8.92%. Found: C, 76.04; H, 9.26%.

9. 3β-Hydroxy-4^1-methyl-5^1,16α-dihydro-5-androsteno[17,16-b] furan 7b

The THP-ether 7a (41 mg) was treated with methanol (3 mL) containing p-TSA (ca 5 mg) at RT for 3 hrs. Work-up with benzene gave the alcohol 7b (31 mg, 94%), mp 266-268°C (acetone-hexane), λ max (EtOH) 223 nm, ε 12200, ν max($CHCl_3$) 3620 (OH), 1750 and 1695 (CO-C=C) cm^{-1}, NMR 0.53 and 1.03 (2s, angular CH$_3$), 1.82 (3H, s, C$_4$1-CH$_3$), 3.55 (1H, m, C$_3$-H), 4.53 (1H, s, C$_{17}$-H), 5.34 (1H, m, C$_6$-H). Mass spectr. for $C_{22}H_{30}O_3$ Calcd. 342.2195. Found: 342.2219.

10. 4^1(R)3β-Hydroxy-4^1-selenophenyl-4^1,5^1,16α,17α-tetrahydro-5-androsteno[17,16-b]furan-5^1-one 3-tetrahydrpyranyl ether 6e

The lactone 6b was treated with phenylselenyl bromide in an analogous manner as the lactone 6c (Experiment 7). The reagents were used as follows: diisopropylamine (98 L, 0.70 mmol) in THF (2 mL), n-butyllithium (0.4 mL of 1.6 M solution in hexane, 0.70 mmol), the lactone 6b (250 mg, 0.60 mmol) in THF (3 mL), phenylselenyl bromide (228 mg, 0.72 mmol), and HMPT (130 L) in THF (2 mL). The crude product was chromatographed on a silica gel column (3 g, elution: hexane - ethyl acetate, 95:5) to give 6e (313 mg, 91%), mp 227-229°(MeOH), ν max (KBr) 3050 (arom.), 1750 (C=O), and 1580 (arom.) cm^{-1}, NMR 0.71 and 0.98 (2s, angular CH$_3$), 3.49 (2H, THP-H), 3.69 (1H, d, J=7 Hz, C$_4$1-H), 3.88 (1H, d, J=10 Hz, C$_{17}$-H), 4.71 (1H, THP-H), 5.30 (1H, m, C$_6$-H), 7.25-7.40 and 7.60-7.75 (aromatic H). Anal. Calcd. for $C_{32}H_{42}O_4Se$: C, 67.49; H, 7.38%. Found: 67.68; H, 7.64%.

11. 4^1(S)3β-Hydroxy-4^1-methyl-4^1-selenophenyl-4^1,5^1,16α,17α-tetrahydro-5-androsteno[17,16-b]furan-5^1-one 3-tetrahydropyranyl ether 6f

The lactone 6e was treated with methyl iodide in an analogous manner as lactone 6b (Experiment 6). The reagents were used as follows: diisopropylamine (81 L, 0.58 mmol) in THF (1.5 mL), n-butyllithium (0.36 mL of 1.6 M in hexane, 0.58 mmol), 6e (283 mg, 0.49 mmol) in THF (2 mL), methyl iodide (80 L, 1.2 mmol), and HMPT (60 L) in THF (1.5 mL).

Chromatography of the crude product on a silica gel column (5 g, elution: hexane - ethyl acetate, 97:3) gave compound 6f (252 mg, 87%), mp 175-177°C (acetone - hexane), ν max (KBr) 3050 (arom.), 1765 (C=O) and 1580 (arom.) cm^{-1}, NMR 0.90 and 1.04 (2s, angular CH$_3$), 3.50 (2H, THP-H), 3.89

(1H, m, C_3-H), 4.36 (1H, d, J=10 Hz, C_{17}-H), 4.71 (1H, THP--H) 5.34 (1H, m, C_6-H), 7.2-7.45 and 7.6-7.8 (aromatic H). Anal.Calcd. for $C_{33}H_{44}O_4Se$: C, 67.92; H, 7.55%. Found: C, 67.79; H, 7.40%.

12. 3β-Hydroxy-4^1-methylidene-4^1,5^1,16α,17α-tetrahydro--5-androsteno[17,16-b]furan-5^1-one 3-tetrahydropyranyl ether 1b

The selenophenyl lactone 6f (70 mg, 0.12 mmol) in THF (2 mL) was treated with 30% hydrogen peroxide (0.2 mL) and acetic acid (0.1 mL) in an analogous manner as described in the Experiment 8. The crude product was chromatographed on a silica gel column (2 g, elution: hexane-ethyl acetate, 95:5) to give compound 1b (48 mg, 93%) mp 193-195°C (acetone-hexane), λ max (MeOH) 211 nm, ε=9900, ν max (KBr) 1760 (C=O) and 1665 (C=C) cm^{-1}, NMR 0.66 and 1.02 (2s, angular CH_3), 3.45 (3H, m, C_{16}-H overlaping THP-H), 3.87 (1H, m, C_3-H), 4.72 (2H, THP-H), 5.33 (1H, m, C_6-H), 5.56 (1H, d, J=3 Hz, C=C-H), 6.18 (1H, d, J=3 Hz, C=C-H). Anal. Calcd. for $C_{27}H_{38}O_4$: C, 76.06; H, 8.92%. Found: C, 75.92; H, 8.55%.

13. 3β-Hydroxy-4^1-methylidene-4^1,5^1,16α,17α-tetrahydro--5-androsteno[17,16-b] furan-5^1-one 1a

The THP-ether 1b (42 mg) was hydrolyzed with p-TSA (ca 2 mg) in methanol (3 mL) for 3 hrs at RT and the product was isolated with benzene to give the alcohol 1a (32 mg, 95%), mp 229-231°C (ethyl acetate), λ max (MeOH) 211 nm, ε=9880, ν max ($CHCl_3$) 3600 (OH), 1760 and 1660 (CO-C=C) cm^{-1}, NMR 0.70 and 1.02 (2s, angular CH_3), 3.50 (2H, m, C_3-H and C_{16}-H), 4.36 (1H, d, J=10 Hz, C_{17}-H), 5.34 (1H, m, C_6-H), 5.55 (1H, d, J=3 Hz, C=C-H), 6.18 (1H, d, J=3 Hz, C=C-H), Mass spect. for $C_{22}H_{30}O_3$ Calcd. 342.2195. Found: 342.2197

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