Absolute configuration at C-24 of 5β-ranol, a principal bile alcohol of the bullfrog

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Abstract The stereochemistry of the hydroxyl group at C-24 in 5β-ranol (27-nor-5β-cholestane-3α,7α,12α,24,26-pentol), a principal bile alcohol of the bullfrog which is structurally related to the major human urinary bile alcohol, 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, is described. Two isomers (IIIa and IIIb) at C-24 of 27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol were synthesized from cholic acid (I) by the conversion to 3α,7α,12α-triacetoxy-5β-cholan-24-al (II) followed by a Grignard reaction with vinylmagnesium bromide. The absolute configurations at C-24 of the unsaturated tetrols (IIIa and IIIb) were elucidated as S and R, respectively, by means of the difference of the reactivity to Sharpless oxidation, a stereoselective epoxidation. Catalytic hydrogenation of each Δ^{25}-tetrol (IIIa or IIIb) gave (24R)- or (24S)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (IVa or IVb). The configurations at C-24 of two isomeric 3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oic acids (Va and Vb) were determined as S and R, respectively, by means of their conversion into the saturated tetrols (IVa and IVb) of known absolute configurations by a Kolbe electrolytic coupling with acetic acid. The lithium aluminum hydride reduction product of the 24R-acid (Vb) was identical with the naturally occurring 5β-ranol, hence 5β-ranol has the 24R configuration. —**Kihira, K., Y. Noma, K. Tsuda, T. Watanabe, Y. Yamamoto, M. Une, and T. Hoshita.** Absolute configuration at C-24 of 5β-ranol, a principal bile alcohol of the bullfrog. *J. Lipid Res.* 1986. **27:** 393–397.

Supplementary key words bile alcohol • absolute configuration

Recent studies have demonstrated that a substantial amount of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol was excreted in human urine (1–5) and bile (6), and that cholesterol is the precursor of this C_{26} bile alcohol (7). Although 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol has not been found in other species, a structurally related C_{26} bile alcohol, 5β-ranol, 27-nor-5β-cholestane-3α,7α,12α,24,26-pentol, has been found in bullfrog bile (8) as its major constituent. The biosynthetic route of 5β-ranol from cholesterol is thought to be identical with that of cholic acid, the most common bile acid in mammals, up to the intermediate 3α,7α,12α-trihydroxy-24-oxo-5β-cholestan-26-oic acid (9–11). The subsequent steps in bullfrog are the decarboxylation of the latter to 3α,7α,12α-trihydroxy-27-nor-5β-cholestan-24-one, reduction to 27-nor-5β-cholestane-3α,7α,12α,24-tetrol, and finally, conversion to 5β-ranol. The mechanism of the formation of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol from cholesterol remains unknown. However, it could be speculated (5) that the 24,25-pentol is formed by a pathway similar to that for the formation of 5β-ranol, in which the only difference is the last step: 25-hydroxylation of the nor-tetrol leads to the former; 26-hydroxylation to the latter.

Although the 24R configuration in 5β-ranol has been assigned by means of the difference of the carbon-13 chemical shift of C-24 in 5β-ranol and its 24-epimer (12), the assignment should be considered tentative, since the side chain of 24-epimeric steroids used as model compounds was quite different from that of 5β-ranol. The former are cholestane-derivatives and have no hydroxyl group at C-26, while the latter is a 27-norcholestane-derivative and has a hydroxyl group at C-26. The possibility that the C_3-terminal isopropyl group in the model compounds and the 26-hydroxyl group in 5β-ranol exert different effects on the chemical shift of C-24 is not fully excluded.

The present study was performed, therefore, in order to determine the absolute configuration at C-24 of 5β-ranol. This was achieved by the configurational assignment of two 24-epimeric 3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oic acids, since it has been known that one of these C_{26} bile acids can be converted to the naturally occurring 5β-ranol (12).

Abbreviations: TLC, thin-layer chromatography; GLC, gas-liquid chromatography; IR, infrared; TMS, trimethylsilyl; RRT, relative retention time; MS, mass spectrometry.

MATERIALS AND METHODS

General

Melting points were determined on a Kofler hot-stage apparatus, and are uncorrected.

Infrared (IR) spectra were obtained on a Shimadzu model IR-408 spectrophotometer as KBr discs. Absorption frequences are quoted in reciprocal centimeters.

Proton nuclear magnetic resonance (PMR) spectra, in δ ppm, were recorded on a Hitachi model R-40 spectrometer at 90 MHz using tetramethylsilane as an internal standard.

Thin-layer chromatography (TLC) was carried out on precoated silica gel G plates (0.25 mm thickness, Merck). The solvent systems used were ethyl acetate–acetone 6:4 (EA-3) for bile alcohols and benzene–isopropanol–acetic acid 30:10:1 (S-7) for bile acids. The spots were detected by spraying with phosphomolybdic acid (10% in ethanol) and heating at 110°C for 5 min.

TLC-direct densitometry was performed using a Shimadzu model CS-910 dual-wavelength chromatoscanner as described previously (13).

Gas–liquid chromatography (GLC) was carried out on a Shimadzu model GC-3BF gas chromatograph equipped with a flame ionization detector. The column used was a glass column (3 mm × 2 m) packed with 3% OV-17 (column temperature 270°C). The samples were separated as their trimethylsilyl (TMS) ether derivatives and the retention times are described relative to that of the TMS ether of methyl cholate (1.00).

Gas–liquid chromatography–mass spectrometry (GLC–MS) was performed on a Shimadzu model QP-1000 gas chromatograph–mass spectrometer using the following conditions: column, WCOT capillary column coated with SE-30 (0.35 mm × 25 m, LKB); column temperature, 265°C; ion source temperature, 250°C.

The phrase, 'the usual workup,' mentioned in the Experimental section refers to extraction with organic solvent, washing to neutrality, drying over anhydrous Na_2SO_4, filtration, and evaporation under reduced pressure.

EXPERIMENTAL

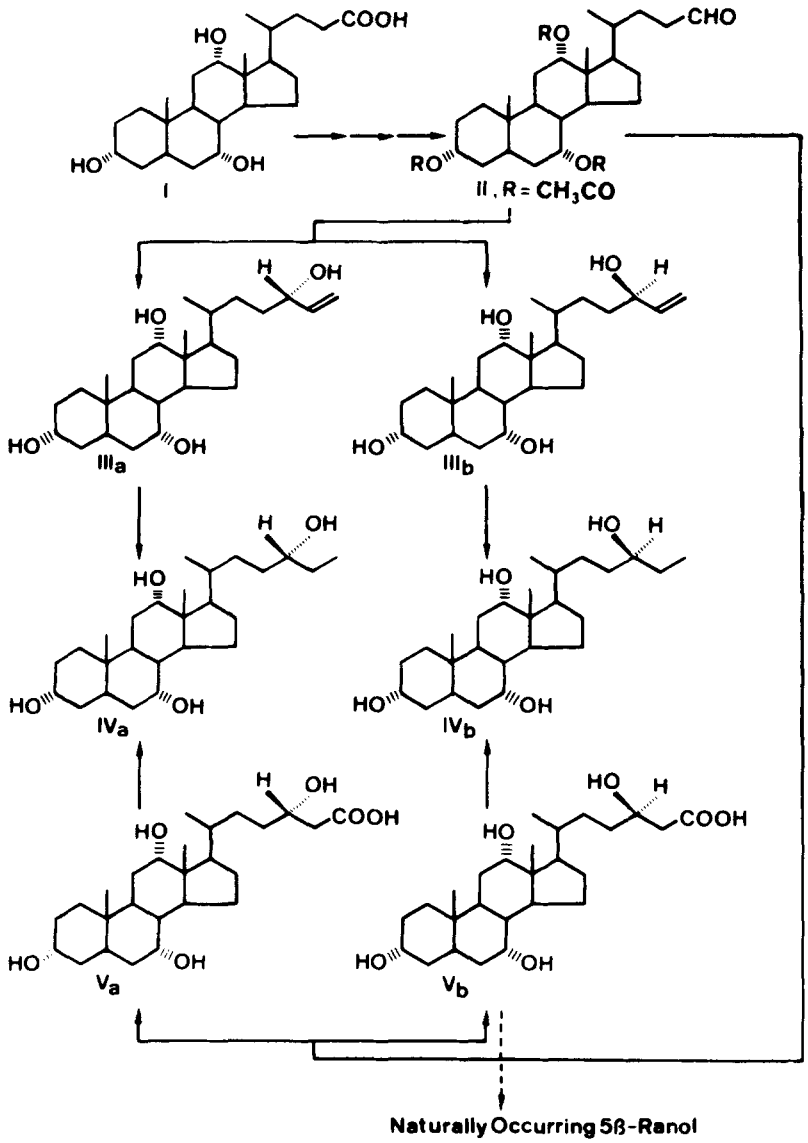
Synthesis of 27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrols (IIIa and IIIb)

3α,7α,12α-Triacetoxy-5β-cholan-24-al (II) (**Scheme 1.**) was prepared from cholic acid (I) according to the method previously reported (14). A solution (50 ml) of 3α,7α,12α-triacetoxy-5β-cholan-24-al (II, 6 g) in anhydrous benzene was added to a tetrahydrofuran solution (150 ml) containing vinylmagnesium bromide prepared from 6 g of magnesium and 25 ml of vinyl bromide. The reaction mixture was stirred for 4 hr at room temperature. After acidification with 1 N HCl, the usual workup (ethyl acetate, 200 ml × 2) gave a residue. The residue was hydrolyzed with 5% methanolic KOH (100 ml) for 2 hr by warming on a water bath (50°C). The reaction mixture was diluted with 5 volumes of water and the usual workup (ethyl acetate, 100 ml × 4) afforded an oily residue. The residue was chromatographed using ethyl acetate graded by acetone as an eluting solvent on a silica gel column (150 g). By monitoring on TLC appropriate fractions containing (24R)- and (24S)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrols were collected and the solvent was removed under reduced pressure. The resulting residue (1.1 g) was rechromatographed on a silica gel column (Lober column LiChroprep Si 60, 310 × 25 mm, 40–63 μm, Merck) using chloroform–acetone–methanol 40:55:5 (by vol) as an eluting solvent. Fractions eluted from 450 ml to 525 ml were collected and the solvent was evaporated. Crystallization of the residue from ethyl acetate gave colorless crystals (280 mg) of (24S)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol (IIIa): mp, 174.5–175.0°C; R_f on TLC (solvent system, EA-3), 0.33; RRT on GLC (as TMS ether, OV-17) 0.88; IR (KBr), 3400 (hydroxyl), 995 and 910 (end methylene); PMR (pyridine-d_5), 0.81 (3H, s, 18–CH_3), 0.98 (3H, s, 19–CH_3), 1.22 (3H, d, J = 6 Hz, 21–CH_3), 3.40–4.50 (4H, m, 3β-, 7β-, 12β-, and 24–H), 5.60 (2H, m, 26–H_2), 6.03 (1H, m, 25–H); mass spectrum (as TMS ether), 528 (M – 90×2, 4.9%), 461 (M – side chain after C-22 – 90, 5.1), 438 (M – 90×3, 6.0), 371 (M – side chain after C-22 – 90×2, 12.8), 348 (M – 90×4, 12.8), 343 (M – side chain – 90×2, 16.6), 281 (M – side chain after C-22 – 90×3, 36.0), 253 (M – side chain – 90 ×3, 45.7), 129 (side chain after C-24, 100).

Fractions eluted from 660 ml to 900 ml were combined and the solvent was evaporated to dryness. Crystallization of the residue from ethyl acetate gave colorless crystals (310 mg) of (24R)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol (IIIb) : mp, 188.0°C; R_f on TLC (solvent system, EA-3), 0.29; RRT on GLC (as TMS ether, OV-17), 0.88; IR (KBr), 3400 (hydroxyl), 995 and 910 (end methylene); PMR (pyridine-d_5), 0.81 (3H, s, 18–CH_3), 0.98 (3H, s, 19–CH_3), 1.22 (3H, d, J = 6 Hz, 21–CH_3), 3.40–4.50 (4H, m, 3β-, 7β-, 12β-, and 24–H), 5.60 (2H, m, 26–H_2), 6.03 (1H, m, 25–H); mass spectrum (as TMS ether), 618 (M–90, 0.7%), 528 (M – 90×2, 4.5), 461 (M – side chain after C-22 – 90, 2.4), 438 (M – 90×3, 6.7), 433 (M – side chain – 90×2, 1.4), 371 (M – side chain after C-22 – 90 ×2, 7.6), 348 (M – 90×4, 11.2), 343 (M – side chain – 90 ×2, 16.0), 281 (M – side chain after C-22 – 90×3, 28.6), 253 (M – side chain – 90×3, 38.4), 129 (side chain after C-24, 100).

Determination of the absolute configuration at C-24 of 27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrols

A 1:1 mixture (50 mg) of the two C-24-epimeric 27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrols (IIIa and IIIb)



Scheme 1. Synthesis and conversion of 27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol and 3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oic acid to 27-nor-5β-cholestane-3α,7α,12α,24-tetrol. I. Cholic acid. II. 3α,7α,12α-triacetoxy-5β-cholan-24-al. IIIa. (24S)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol. IIIb. (24R)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol. IVa. (24R)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol. IVb. (24S)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol. Va. (24S)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid. Vb. (24R)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid.

was added to a solution prepared with 1.02 ml of tetraisopropyl orthotitanate and 10 ml of L-(+)-tartaric acid diisopropyl ester in 10 ml of tetrahydrofuran. After stirring for 5 min, 6 ml of *tert*-butyl hydroperoxide was added and the reaction mixture was stirred for 3 hr at room temperature under a stream of argon. The mixture was diluted with water (100 ml) and the usual workup with ethyl acetate (80 ml × 3) gave a residue. The residue was subjected to TLC–direct densitometry (solvent system, EA-3) to determine the amounts of the unreacted tetrols (IIIa and IIIb). Measurements of the color intensities of the tetrol spots revealed that the residue contains the less polar isomer (IIIa) and the more polar isomer (IIIb) in the ratio of 0.48 : 1.00.

27-Nor-5β-cholestane-3α,7α,12α,24-tetrols, (IVa and IVb) from 27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrols (IIIa and IIIb)

A solution of (24S)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol (IIIa, 10 mg) dissolved in 10 ml of methanol was hydrogenated with PtO_2 (10 mg) as the catalyst at room temperature. After removal of the catalyst from the reaction mixture by filtration, the solvent was evaporated to dryness. Recrystallization of the residue gave crystals of

(24R)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (IVa): mp, 191°C; R_f on TLC (solvent system, EA-3), 0.33; RRT on GLC (as TMS ether, OV-17) 0.89; PMR (pyridine-d_5), 0.79 (3H, s, 18-CH_3), 0.99 (3H, s, 19-CH_3), 1.15 (3H, t, J = 6 Hz, 26-CH_3), 1.27 (3H, d, J = 6 Hz, 21-CH_3), 3.5-4.4 (4H, m, 3β-, 7β-, 12β-, and 24-H); mass spectrum (as TMS ether), 530 (M - 90×2, 14.9%), 515 (M - 90×2 - CH_3, 1.3), 501 (M - side chain after C-25 - 90×2, 1.2), 440 (M - 90×3, 28.2), 433 (M - side chain - 90, 1.7), 425 (M - CH_3 - 90×3, 2.2), 411 (M - side chain after C-25 - 90×3, 3.3), 351 (M - 90×4 + H, 21.7), 343 (M - side chain - 90×2, 39.7), 335 (M - 90×4 - CH_3, 8.3), 321 (M - side chain after C-25 - 90×4, 9.7), 281 (M - side chain after C-22 - 90×3, 17.4), 253 (M - side chain - 90×3, 67.0), 131 (side chain after C-24, 100).

(24R)-27-Nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol (IIIb, 20 mg) was hydrogenated as described for IIIa and gave crystals of (24S)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (IVb): mp, 181-183°C; R_f on TLC (solvent system, EA-3), 0.29; RRT on GLC (as TMS ether, OV-17) 0.89; PMR (pyridine-d_5), 0.84 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.17 (3H, t, J = 6 Hz, 26-CH_3), 1.28 (3H, d, J = 6 Hz, 21-CH_3), 3.5-4.4 (4H, m, 3β-, 7β-, 12β-, and 24-H); mass spectrum (as TMS ether), 530 (M - 90×2, 19.7%), 515 (M - 90×2 - CH_3, 1.4), 501 (M - side chain after C-25 - 90×2, 1.5), 440 (M - 90×3, 33.8), 433 (M - side chain - 90, 1.8), 425 (M - CH_3 - 90×3, 2.5), 411 (M - side chain after C-25 - 90×3, 3.8), 351 (M - 90×4 + H, 22.2), 343 (M - side chain - 90×2, 39.7), 335 (M - 90×4 - CH_3, 8.2), 321 (M - side chain after C-25 - 90×4, 10.9), 281 (M - side chain after C-22 - 90×3, 18.2), 253 (M - side chain - 90×3, 66.5), 131 (side chain after C-24, 100).

27-Nor-5β-cholestane-3α,7α,12α,24-tetrols, (IVa and IVb) from 3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oic acids (Va and Vb)

Two epimers (Va and Vb) at C-24 of 3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oic acids were prepared from 3α,7α,12α-triacetoxy-5β-cholan-24-al (II) according to the method previously reported (12).

Acetic acid (5 ml) and 100 mg of the less polar 3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oic acid (Va)[mp, 183-184°C (as methyl ester); R_f on TLC (solvent system, S-7), 0.42] was added to a methanol solution (200 ml) containing 500 mg of sodium. The solution was electrolyzed for 5 hr with two platinum electrodes using direct current from a 30 V source at 1 A current keeping the reaction temperature at 20-25°C by external cooling. After the usual workup (extraction with ethyl acetate), the product was analyzed by TLC with solvent system EA-3 and shown to be identical with (24R)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (IVa).

By the same procedure, the more polar isomer (Vb)[mp, 162-163°C (as methyl ester); R_f on TLC (solvent system, S-7), 0.36] gave a product which was analyzed by TLC with solvent system EA-3 and shown to be identical with (24S)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (IVb).

RESULTS AND DISCUSSION

Two isomers (IIIa and IIIb) at C-24 of 27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrol were synthesized from 3α,7α,12α-triacetoxy-5β-cholan-24-al (II), which was prepared from cholic acid (I), by a Grignard reaction with vinylmagnesium bromide (14). The resolution of these isomers was achieved by using a prepacked silica gel column (Lober column LiChroprep Si 60, 40-60 μm, Merck). The 1:1 mixture of IIIa and IIIb was epoxidized by Sharpless oxidation which is known as a reliable method not only for stereoselective introduction of a hydroxyl group (15) but also for resolution of the epimeric pairs of allyl alcohols (16). After treatment with *t*-butyl hydroperoxide in the presence of L-(+)-tartrate and tetraisopropyl orthotitanate, the ratio of the remaining tetrols (IIIa and IIIb) was changed to 0.48:1 from 1:1 indicating that only the less polar isomer (IIIa) was epoxidized. It has been known that, under the conditions employed here, only the 24S-isomer could be epoxidized while the 24R-isomer remains unreacted (16). Therefore, the epoxidizable, less polar isomer (IIIa) was assigned the 24S configuration and the more polar one (IIIb) the 24R configuration.

The two unsaturated tetrols (IIIa and IIIb) of known absolute configuration at C-24 were individually hydrogenated using platinum oxide as the catalyst. The less polar 24S-isomer (IIIa) gave the less polar saturated tetrol, (24R)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (IVa), and the more polar isomer (IIIb) gave the more polar tetrol, (24S)-27-nor-5β-cholestane-3α,7α,12α,24-tetrol (IVb).

Two isomers (Va and Vb) at C-24 of 3α,7α,12α,24-tetrahydroxy-27-nor-5β-cholestan-26-oic acid were synthesized and separated as described previously (12). The configurations at C-24 of Va and Vb were deduced by the conversion of each C_{26} bile acid into the (24R)- or (24S)-norcholestanetetrol (IVa or IVb) of known absolute configuration. Kolbe electrolytic coupling with acetic acid of the less polar acid (Va) gave the less polar 24R-tetrol (IVa). By the same procedure, the more polar acid (Vb) gave the more polar 24S-tetrol (IVb). Thus the absolute configuration at C-24 of the less polar 3α,7α,12α,24-tetrahydroxy-27-nor-5-cholestan-26-oic acid (Va) was deduced as 24S, and the more polar counterpart as 24R. Since it has been known that the more polar C_{26} bile acid (Vb) can be converted into the naturally occurring 5β-ranol by lithium aluminum hydride reduction (12), it can

be concluded that 5β-ranol has the 24R configuration. This assignment confirmed the previous tentative assignment by carbon 13 nuclear magnetic resonance spectroscopy (12).

Recently, Dayal et al. (17) have reported that the 24S configuration of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol excreted in urine and feces from a patient with sitosterolemia and xanthomatosis was assigned by means of lanthanide-induced circular dichroism measurements (Cotton effect). Hence, 5β-ranol and the 24,25-pentol have the opposite configuration at C-24. The most likely explanation is that, in the bullfrog, only the (24S)-27-nor-5β-cholestanetetrol (IVb) is hydroxylated at C-26, while in man the hydroxylation of the 24R-counterpart (IVa) occurs at C-25. It is known that 5β-ranol is an efficient precursor of cholic acid in bile fistula rats (18), but the metabolic role of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol as a precursor of cholic acid in man is unknown. In order to study the metabolism of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol, preparation of four diastereoisomers at C-24 and C-25 of the C_{26}-pentol is required, since it is known that only the (24R)-5β-cholestane-3α,7α,12α,24,25-pentol (but not the 24S-counterpart) could be converted to cholic acid in man (19). The stereospecific synthesis of all four isomers of 27-nor-5β-cholestane-3α,7α,12α,24,25-pentol may be accomplished by the stereoselective epoxidation of (24R)- and (24S)-27-nor-5β-cholest-25-ene-3α,7α,12α,24-tetrols prepared here followed by the reduction with lithium aluminum hydride. The detail of such studies will be reported in a later paper.

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