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New Metabolites from the South African Soft Coral *Capnella thyrsoidea*

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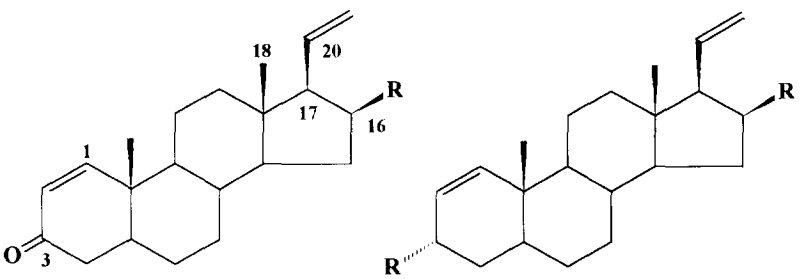
Abstract: Ethyl acetate extracts of two phenotypic color variants of the endemic South African soft coral, *Capnella thyrsoidea* yielded the known steroid 5α-pregna-1,20-dien-3-one (**1**) and a further six new compounds, 16ß-hydroxy-5α-pregna-1,20-dien-3-one 16-acetate (**2**), 3α,16ß-dihydroxy-5α-pregna-1,20-diene 3,16-diacetate (**3**) and four xenicane diterpenes, the tsitsixenicins A-D (**6**, **10** - **12**). Standard spectroscopic methods were used to determine the structures and stereochemistry of these compounds. This is the first reported isolation of xenicane diterpenes from the soft coral family Nephtheiidae. Compounds **6** and **10** inhibit superoxide production in both rabbit and human cell neutrophils.

Coastal southern Africa, stretching from the Skeleton Coast in Namibia to Beira in Mozambique, has an extremely rich and diverse marine invertebrate fauna. Octocorals form a major invertebrate component of the benthic reef communities off the southern African coast and of the approximately 200 species occurring in this region 60-70% are known to be endemic.[1] Soft corals (Order Alcyonacea) are one of the six orders found within the class Octocorallia and although they are generally brightly coloured and rich in nutritionally important substances, the incidence of predation in the majority of these organisms is low because of the toxic terpenoid compounds they produce. Some of these terpenoids have shown potential as possible pharmaceuticals and recently a South African soft coral, *Alcyonium valdivae*, proved to be a good source of new anti-inflammatory diterpenes.[2] Therefore, in continuation of our search for new bioactive natural products from benthic marine invertebrates collected with SCUBA in the Tsitsikamma Marine Reserve,[3] we have examined the endemic soft coral, *Capnella thyrsoidea* (Family Nephtheiidae). This is one of the most common octocorals found off the South African coast and has several phenotypic color variants.[4] In this paper we report the isolation and identification of seven pregnadiene steroid and xenicane diterpene metabolites from clearly distinguishable bright yellow and grey color variants of *C. thyrsoidea*.

Separate ethyl acetate extracts of the freeze dried yellow (496g) and grey (206g) color variants of *C. thyrsoidea* were concentrated under reduced pressure to give dark brown oils (14g and 5g respectively). These were screened by ^{1}H NMR and although the ^{1}H NMR spectra were similar, significant differences in the series of peaks occurring between δ 4.8 and 7.2 precluded the combining of these two extracts. Flash chromatography of portions of each extract on silica gel using a hexane/ethyl acetate solvent gradient gave several fractions with interesting ^{1}H NMR spectra. A group of chromatography fractions from the yellow color variant extract contained predominantly

a single compound as judged from TLC and ^{1}H NMR spectroscopy. These fractions were combined, concentrated and purified further by HPLC to give a crystalline residue. This was recrystallised from hexane to give white needles of (**1**, mp 125-126°C; $[\alpha]_D$ +46°) which was found to be identical, by comparison of the spectral data, melting point and optical rotation, with 5α-pregna-1,20-dien-3-one previously isolated from an Australian *Capnella* species, *C. erecta*,[5] and an unidentified soft coral from Canton Island.[6] This compound represents 0.13% of the dry weight of the yellow color variant of *C. thyrsoidea*.



1 R = H
2 R = OAc
4 R = OH

3 R = OAc
5 R = OH

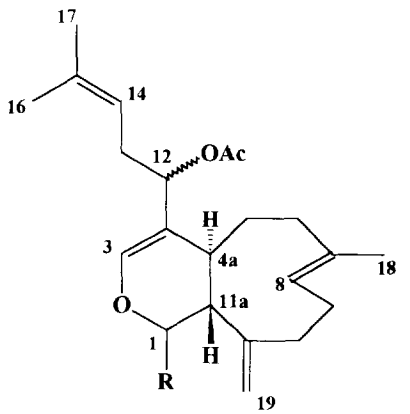
Characteristic ^{1}H NMR signals for pregna-1,20-dien-3-one compounds include two doublets at δ 7.13 and 5.83 ($J_{1,2}$ = 10 Hz, H-1 and H-2), a triplet of doublets at δ 5.74 (H-20) and a two proton mutiplet at δ 4.95 (H_2-21), and can be used to identify these compounds in the ^{1}H NMR spectra of crude extracts or chromatography fractions. No further pregna-1,20-dien-3-ones or similar compounds were evident from the ^{1}H NMR spectra of the remaining yellow color variant chromatography fractions. However, the ^{1}H NMR spectra of two fractions from the flash chromatography of the grey color variant revealed the presence of pregna-1,20-diene compounds and both fractions were concentrated separately to give two yellow oils. Further chromatography of these oils yielded two new compounds **2** (0.009% dry wt.) and **3** (0.006% dry wt.) as optically active colourless oils, $[\alpha]_D$+91° and +133° respectively. Although there was no evidence of **1** in any of the chromatography fractions from the grey color variant of *C. thyrsoidea*, biosynthetic arguments and the common spectral data exhibited by compounds **1** - **3** suggested a 5α-pregnadiene parent structure for compounds **2** and **3**.

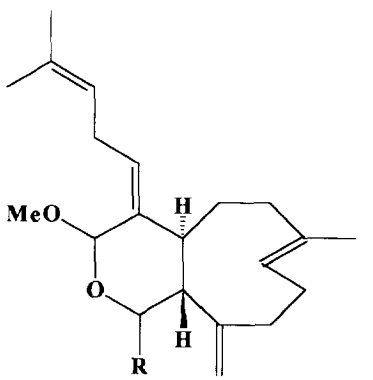
A molecular formula of $C_{23}H_{32}O_3$ (m/z 356.2338, Δmmu -13) from HREIMS data and an acetate methyl singlet at δ 2.02 in the ^{1}H NMR spectrum of 16ß-hydroxy-5α-pregna-1,20-dien-3-one 16-acetate (**2**), confirmed that this compound was a monoacetoxy derivative of **1**. The position of the acetoxy group at C-16 followed from the coupling observed between H-16 (δ 5.11, m, 1H) and H-17 (δ 2.15, dd, $J_{17,20}$ = 7.7 Hz, $J_{16,17}$ = 4.1 Hz, 1H) in the COSY spectrum of **2**. The 16ß stereochemistry of this functionality was suggested from selected NOEDS experiments. Irradiation of the H-17 signal resulted in significant enhancement of the H-16 signal while irradiation of H-16 caused enhancement of H-17 and H-15α. Saponification of **2** gave 16ß-hydroxy-5α-pregna-1,20-dien-3-one (**4**), as a yellow oil, $[\alpha]_D$ +12°. The γ-*gauche* effect of the hydroxy group(s) in hydroxylated steroid molecules is a useful tool for the structure elucidation of these compounds[7] and provided further evidence for the 16ß

configuration of the acetoxy group in **3**. A γ-hydroxy substituent effect, arising from the 16ß-hydroxy group, causes an upfield chemical shift (-3 ppm) of the C-20 signal in the ^{13}C NMR spectrum of **4** relative to **1**. In addition, the ^{13}C chemical shift of the C-18 methyl group, which is nearly 1,3-synperiplanar to the 16ß-hydroxy group, is affected by a small δ-hydroxy substituent effect and is shifted marginally downfield (+1 ppm).[7]

HREIMS established the molecular formula of the third pregnadiene compound, 3α,16ß diacetoxy-5α-pregna-1,20-diene (**3**), as $C_{25}H_{36}O_4$ (m/z 400.2609, Δmmu -4). The two acetate methyl proton singlets in the ^1H NMR spectrum of **3** (δ 2.02 and 2.04) and the similarities observed between the spectral data of **3** and compounds **1** and **2** confirmed the diacetoxy 5α-pregna-1,20-diene structure of this compound. The absence of the characteristic α,ß-unsaturated carbonyl signal at δ 200 in the ^{13}C NMR spectrum of **3** and the coupling observed between the oxymethine proton H-3 (δ 5.15, m, 1H) and the vicinal protons, H-2 (5.60, ddd, $J_{1,2}$ = 10 Hz, $J_{2,3}$ = 5.5 Hz, $J_{2,4}$ = 1.2 Hz, 1H) and H-4a, H-4e (δ 1.53 and 1.81, m, 2H), in the COSY spectrum of this compound unequivocally placed the one acetoxy group at C-3. LAH reduction of **3** gave the diol **5**, as a white solid, $[\alpha]_D$ - 63°. The ^1H NMR spectrum of **5** contained a broad triplet (δ 4.1, $W_{½}$ = 10Hz)[6] assigned to the 3ß proton (a 3α proton requires $W_{½}$ = 25Hz)[8] thus confirming the α configuration of the oxygen functional group at C-3. The 16ß position and stereochemistry of the second acetoxy group in **3** was established using the same techniques and associated arguments presented earlier for **2**. A combination of COSY, HMQC and HMBC NMR experiments enabled complete assignment of the ^{13}C chemical shifts of compounds **1** - **5** (see Table 1).

A series of diterpene compounds were also isolated during the chromatographic purification of compounds **1** - **3**. Further HPLC chromatography of a fraction obtained during the HPLC purification of **1** from the yellow color variant extract, gave the diterpene tsitsixenicin A (**6**, 0.013% dry wt.) as a yellow oil, $[\alpha]_D$- 64°. The molecular formula of **6** was determined from HREIMS data as $C_{24}H_{34}O_5$ (m/z 400.2398, Δmmu -8). The two acetate carbonyl





6 R = α-OAc
7 R = β-OAc

8 R = α-OAc
9 R = β-OAc

(δ 169 and 170) and eight olefinic (δ 113, 116, 119, 124, 134, 136, 143 and 149) resonances in the ^{13}C NMR spectrum of **6** accounted for six of the eight degrees of unsaturation implied by the molecular formula. The remaining two degrees of unsaturation therefore required a bicyclic diterpene skeleton for this compound.

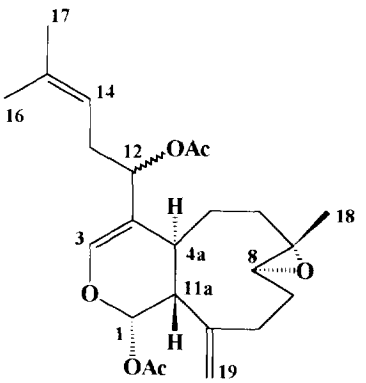
The ^{1}H and ^{13}C NMR data of **6**, supported by an HMQC NMR experiment, revealed the presence of one acetylated hemiacetal [δ_H 5.75 (d, $J_{1,11a}$ = 3.6 Hz, 1H, H-1) and δ_C 92], an olefinic methylene group [δ_H 4.78 and 4.91 (brs, 2H, H-19, H-19') and δ_C 113], three vinylic methyl groups [δ_H 1.64 (brs, 3H, H_3-16), 1.67 (brs, 6H, H_3-17, H_3-18) and δ_C 17, 18 and 25] and an enol ether methine proton [δ_H 6.52 (d, $J_{3,4a}$ = 2 Hz, 1H, H-3) and δ_C 143]. The contiguous coupling sequence observed in the COSY spectrum of **6** from the hemiacetal proton (H-1) through

Table 1. ^{13}C [100MHz, $CDCl_3$, δ ppm (mult.)] data for compounds **1** - **5**.

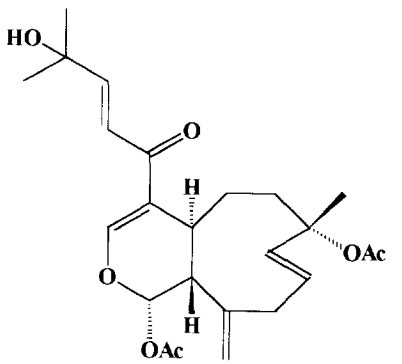
C#	1	2	3	4	5
1	158.5 (d)	158.0 (d)	142.0 (d)	158.2 (d)	140.3 (d)
2	127.4 (d)	127.5 (d)	122.5 (d)	127.5 (d)	126.2 (d)
3	200.1 (s)	200.0 (s)	67.3 (d)	200.1 (s)	64.4 (d)
4	40.1 (t)	40.1 (t)	31.8 (t)	41.0 (t)	31.9 (t)
5	44.4 (d)	44.4 (d)	39.6 (d)	44.4 (d)	39.0 (s)
6	27.2 (t)	27.5 (t)	27.6 (t)	27.6 (t)	27.9 (t)
7	31.4 (t)	31.2 (t)	31.7 (t)	31.3 (t)	29.7 (t)
8	35.8 (d)	35.3 (d)	35.3 (d)	35.6 (d)	35.4 (d)
9	50.3 (d)	50.3 (d)	51.0 (d)	50.3 (d)	51.3 (d)
10	39.1 (s)	39.1 (s)	37.9 (s)	39.9 (s)	38.3 (s)
11	27.6 (t)	33.8 (t)	33.9 (t)	35.3 (t)	34.8 (t)
12	37.4 (t)	37.2 (t)	37.3 (t)	37.3 (s)	35.7 (t)
13	43.7 (s)	44.2 (s)	44.2 (s)	44.9 (s)	45.0 (s)
14	55.6 (d)	53.3 (d)	53.4 (d)	53.2 (d)	53.4 (d)
15	20.8 (t)	20.4 (t)	20.2 (t)	20.4 (t)	20.3 (t)
16	24.7 (t)	78.4 (d)	78.5 (d)	76.5 (d)	76.6 (d)
17	55.3 (d)	61.6 (d)	61.6 (d)	66.2 (d)	66.3 (d)
18	13.0 (q)	14.2 (q)	14.2 (q)	14.4 (q)	14.4 (q)
19	13.0 (q)	13.1 (q)	13.9 (q)	13.1 (q)	13.9 (q)
20	139.5 (d)	135.9 (d)	136.1 (d)	136.8 (d)	136.9 (d)
21	114.7 (t)	117.0 (t)	116.8 (t)	117.2 (t)	116.9 (t)
CO*CH*$_3$		21.2 (q)	21.3 (q)		
			21.5 (q)		
***CO*CH$_3$**		171.1 (s)	171.1 (s)		
			170.1 (s)		

the methine protons H-11a (δ 1.96, brs, 1H) and H-4a (δ 2.29, m, 1H) and finally through weak allylic coupling to the enol ether proton (H-3) suggested that one of the rings in the bicyclic structure was a 1-acetoxydihydropyran moeity. Prominent correlations observed in the HMBC spectrum of **6**, between H-3 and C-1, C-4, C-4a further supported this structural assignment. The 2D NMR spectra of **6** also indicated the presence of a 1-acetoxy-4-methyl-pent-3-ene fragment which was placed at C-4 from HMBC correlations between the acetoxy methine proton (δ 5.25, t, $J_{12,13}$ = 7 Hz, H-12) and C-3, C-4. The 1-acetoxydihydropyran ring system and associated six carbon side chain is characteristic of xenicane diterpenes containing a 2-oxabicyclo[7.4.0]undecane structure. The remaining protons and carbon atoms could thus be assigned to a nine membered ring with an exocyclic methylene functionality and a vinylic methyl group from the 2D-NMR data and by comparison of these spectral data with published values for these compounds.[9-13]

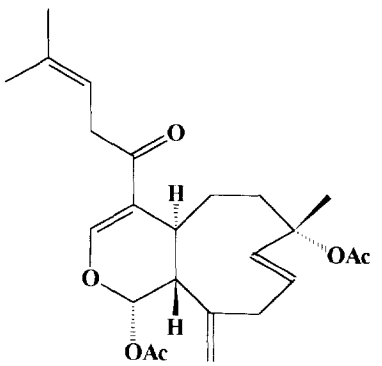
The slightly broadened H-11a singlet at δ 1.96 in the ^{1}H NMR spectrum of **6** required the coupling to H-4a to be very small (a dihedral angle of approximately 90°) and is in accordance with the *trans*-fused bicyclic ring system found in other naturally occuring xenicane diterpenes.[10] The coupling of 3.6 Hz between H-1 and H-11a is consistent with that normally encountered (1.5-5.5 Hz) for an α proton at C-1, the prevalent stereochemistry at this



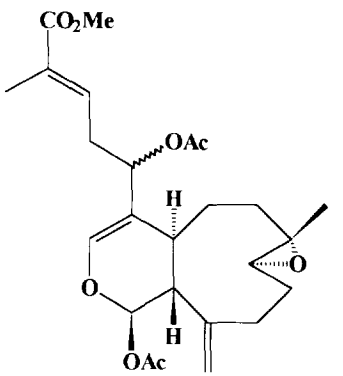
10



11



12



13

position in analogous 1-acetoxy-xenicane compounds.[9-13] However, NOEDS experiments tentatively suggested a ß relative configuration for H-1 in tsitsixenicin A. Irradiation of the H-11a signal only gave significant enhancement (12%) of the H-1 signal while irradiation of H-1 gave enhancement of H-11a (5%) and no enhancement of the H-4a signal. This latter enhancement is evident in NOEDS experiments with xenicane compounds containing a H-1α relative stereochemistry.[11] 9-Deacetoxy-14,15-deepoxyxeniculin (**7**) isolated from the Red Sea soft coral *Xenia macrospiculata* is diastereomeric with **6**.[12] Unfortunately no optical rotation was reported for **7** where the relative stereochemistry at C-1, C-4a and C-11a was assigned from comparison of ^{13}C chemical shifts with analogous compounds of known stereochemistry. The C-1 ^{13}C chemical shift in **6** differs marginally from that reported for **7** (see Table 2) and the assignment of stereochemistry from ^{13}C chemical shift comparisons must therefore be considered tenuous in these compounds.

Tsitsixenicin A was also isolated from the grey color variant extract (0.12% dry wt.) and found to be identical (NMR, $[\alpha]_D$) with **6** obtained from the yellow color variant. Gentle refluxing of a methanolic solution of **6** gave compound **8**, $[\alpha]_D$ -95°, in almost quantitative yield. A similar compound, acalycigorgin D (**9**, $[\alpha]_D$ -51°), recently isolated from the gorgonian *Acalycigorgia* sp.,[11] was assumed to be an artifact arising from **7** via a S_N2' syn reaction with the methanol extraction solvent. From the ease by which this nucleophilic substitution reaction was effected with **6**, it is not surprising that no compound **7** was obtained from the gorgonian extract. Fortuitously, only ethyl acetate was used to extract both color variants of *C. thyrsoidea*. Although no other xenicanes were evident in the yellow color variant extract a combination of exhaustive reverse and normal phase semi prep. HPLC of selected chromatography fractions of the grey color variant extract, yielded a further three new xenicane diterpenes tsitsixenicin B (**10**, 0.006% dry wt.), tsitsixenicin C (**11**, 0.007% dry wt.) and tsitsixenicin D (**12**, 0.008% dry wt.) as viscous, colorless oils.

The molecular ion at m/z 418.2378 (Δmmu +23) in the HREI mass spectrum of tsitsixenicin B is compatible with the molecular formula $C_{24}H_{34}O_6$ and this corresponds to the addition of a single oxygen atom to **6**. The ^{13}C NMR data of **10** (see Table 2) were consistent with that of **6** except for the replacement of the Δ^7-trisubstituted double bond with an oxirane ring (δ 59 and 62). A DEPT NMR experiment confirmed the quarternary character of the former epoxy carbon atom while a HMQC NMR experiment assigned the prominent oxymethine doublet of doublets (δ 2.9, J = 3.2, 10.8 Hz) in the ^1H NMR spectrum, to the latter. The position of the epoxy methine proton at C-8 was established by comparison of the spectral data of **10** with that reported for acalycigorgan B (**13**).[11] Two and three bond correlations between the C-18 methyl protons and C-7 and C-8 respectively in the HMBC NMR spectrum of tsitsixenicin B provided further evidence for the position of the epoxide functionality. The observed nOes between 8-H and the α-proton at C-4a and between the C-18 methyl protons and the ß proton at C-11 in a series of NOEDS experiments performed on **10**, are in accordance with observations reported for the α-orientated oxirane ring in **13**.[11] The stereochemistry at C-12 in tsitisixenicin A and B remains unassigned.

HREIMS established the molecular formulae of tsitsixenicins C and D as $C_{24}H_{32}O_7$ (m/z 432.1961, Δmmu +13) and $C_{24}H_{32}O_6$ (m/z 416.2174, Δmmu -25) respectively. The ^{13}C NMR data of compounds **11** and **12** (see Table 2) indicated the same basic xenicane diterpene skeleton established for compounds **6** and **10** with the differences confined to the six carbon side-chain at C-4 and the substitution pattern around the nine membered ring. One of the two acetoxy groups, evident from the ^1H and ^{13}C NMR spectra of compounds **11** and **12**, was situated at C-1 (δ_C 93) while the second acetoxy group was placed at C-7 from an HMBC correlation between the C-18 methyl protons (δ 1.61) and the quarternary, oxygenated carbon atom (δ 84, C-7). The Δ^8 position of the endocyclic

olefin in **11** and **12** followed from a further HMBC correlation between the vinylic proton at δ 5.40 (d, $J_{8,9}$ = 11.9 Hz, H-8) and C-7. Irradiation of the 18-methyl protons in **11** and **12** induced significant nOe enhancement of H-8 and marginal enhancement of H-11a suggesting a tentative α-configuration for the acetoxy group at C-7. The significant nOe enhancement of the H-11a signal on irradiation of H-1 in compounds **10** - **12** confirmed that the configuration at C-1 was consistent with that proposed for **6**.

Table 2. ^{13}C [100MHz, $CDCl_3$, δ ppm (mult.)] data for compounds **6**, **7*** and **10** - **12**.

C#	6	7	10	11	12
1	92.3 (d)	91.8 (d)	92.0 (d)	93.4 (d)	93.3 (d)
3	142.6 (d)	140.7 (d)	142.4 (d)	151.7 (d)	151.2 (d)
4	116.1 (s)	115.9 (s)	116.2 (s)	124.3 (s)	123.5 (s)
4a	39.7 (d)	36.7 (d)	37.0 (d)	28.2 (d)	28.6 (d)
5	32.2 (t)	30.5 (t)	31.2 (t)	27.0 (t)	26.9 (t)
6	39.7 (t)	40.0 (t)	39.2 (t)	37.5 (t)	37.8 (t)
7	134.4 (s)	134.3 (s)	59.8 (s)	83.7 (s)	83.5 (s)
8	124.3 (d)	124.3 (d)	62.3 (d)	131.2 (d)	131.4 (d)
9	25.5 (t)	25.0 (t)	25.3 (t)	129.8 (d)	129.7 (d)
10	35.9 (t)	35.4 (t)	31.2 (t)	30.7 (t)	30.8 (t)
11	149.3 (s)	151.2 (s)	146.8 (s)	145.5 (s)	145.6 (s)
11a	50.1 (d)	49.3 (d)	49.3 (d)	48.1 (d)	48.0 (d)
12	74.7 (d)	74.9 (d)	74.3 (d)	190.2 (s)	197.7 (s)
13	31.4 (t)	31.3 (t)	31.3 (t)	125.6 (d)	38.6 (t)
14	118.9 (d)	119.0 (d)	118.6 (d)	148.4 (d)	117.0 (d)
15	135.6 (s)	135.8 (s)	134.7 (s)	82.1 (s)	135.0 (s)
16	18.1 (q)	18.1 (q)	18.1 (q)	24.2 (q)†	25.8 (q)
17	25.7 (q)	25.7 (q)	25.7 (q)	24.1 (q)†	18.1 (q)
18	17.0 (q)	16.7 (q)	17.2 (q)	28.5 (q)	28.5 (q)
19	113.4 (t)	113.1 (t)	116.2 (t)	118.1 (t)	117.9 (t)
COCH$_3$					
	21.5 (q)	21.3 (q)	21.4 (q)	22.3 (q)	22.2 (q)
	21.0 (q)	20.9 (q)	21.0 (q)	20.9 (q)	20.9 (q)
***CO*CH$_3$**					
	169.5 (s)	170.2 (s)	170.2 (s)	169.2 (s)	169.3 (s)
	170.2 (s)	169.6 (s)	169.3 (s)	169.2 (s)	169.1 (s)

* Data recorded at 54MHz in $CDCl_3$[12] † Signals may be interchangeable

The spectral data of tsitsixenicin C and D suggested that the differences between these two compounds were limited to the side chain at C-4. The resonance at δ_C 82 and a hydroxyl absorbance (3620 cm^{-1}) in the IR spectrum of **11** indicated the presence of a tertiary hydroxyl group in this compound. This functionality was placed at C-15 in **11** from the HMBC correlations observed between the C-16 and C-17 methyl group protons (δ 1.40 and 1.39) and the carbon signal at δ_C 82. Other HMBC correlations between the two vinylic proton signals (δ 6.82, d, $J_{13,14}$ = 15.8 Hz, H-14 and δ 6.51, d, $J_{13,14}$ = 15.8 Hz, H-13) and the two carbon resonances at δ 82 (C-15) and δ 190 (C-12) respectively, established the 1-keto-4-hydroxy-4-methyl-pent-2-ene structure of the C-4 side chain in **11**. The coupling constant (16 Hz) of the vinylic protons in the side chain supported an E-stereochemistry for the exocyclic olefin in tsitsixenicin C. The 1-keto-4-methyl-pent-3-ene structure of the C-4 side chain in **12** was similarly determined from 2D NMR data. A prominent HMBC correlation between H-3 and C-12 further supported the position of the ketone functionality in the side chain in both compounds **11** and **12** and linked the side chain to the 1-acetoxy-dihydropyran ring as expected.

Although mostly found in soft corals of the genera *Xenia*[9] and *Anthelia*[10] (Family Xeniidae), xenicane diterpenes with the basic skeleton of 7 have also been isolated recently from the soft coral family Helioporidae[13] and a number of gorgonians of the genus *Acalycigorgia*.[11,14] However these compounds have never been reported from the soft coral family Neptheiidae before and the tsitsixenicins A-D represent the first examples of xenicane diterpenes in this family. In a recent review of the chemistry and chemical ecology of octocorals the distribution of terpenes in the genus *Capnella* was reported as being limited to sesquiterpenes.[15] The discovery of xenicanes in *C. thyrsoidea* is therefore at variance with this broad generalisation and further chemotaxonomic investigation of other phenotypic variants within this Southern African species is necessary.

Superoxides (O_2^-) are the precursors of various lethal oxidants (H_2O_2 and HOCl) and are implicated in the biosynthesis of prostaglandins from arachidonic acid at the onset of tissue inflammation. Examination of superoxide inhibition by natural products is therefore one way of discovering new anti-inflammatory drugs. The excess oxygen, consumed by stimulated rabbit and human cell neutrophils, is reduced to superoxides by plasma membrane bound NADPH oxidase. It is possible to assay the concentration of this superoxide through its reduction of cytochrome c, a process that can be monitored by visible spectrophotometry. Compounds **6**, **8**, **10** and **12** showed good inhibition (> 80%) of superoxide production in rabbit neutrophils at a concentration of 12.5 $\mu g ml^{-1}$ with only compound **10** retaining this level of activity on ten fold dilution. The results obtained from the rabbit and human cell neutrophil bioassays often differ. Human cell neutrophils appear less active than rabbit cell neutrophils and the superoxide inhibition by compounds in the former neutrophils is generally reduced.[16] Accordingly, only compounds **6** and **10** showed good to moderate inhibition (68% and 21% respectively) of superoxide production in the human neutrophil bioassay at low concentrations (1.25 $\mu g\ ml^{-1}$). Interestingly, compounds **1** and **2** stimulated superoxide production in rabbit neutrophils and this observation is attributed to cytotoxicity in which cell lysis increases superoxide levels.

Experimental Section

Infrared spectra were recorded on a Perkin Elmer series 7 FTIR spectrophotometer. The ^1H (400MHz) and ^{13}C (100MHz) NMR spectra were recorded on a Bruker AMX400 spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Low resolution mass spectra were recorded on a Hewlett-Packard 5988A

spectrometer and high-resolution spectra were obtained by Dr P. Boshoff of the Mass Spectrometry Unit at the Cape Technikon, Cape Town. High-performance liquid chromatographic separations were performed on Whatman Magnum 9 Partisil and Phenomenex Selectosil C-18 columns.

Collection, extraction and isolation: Yellow and grey color variants of the soft coral *Capnella thrysoidea* were collected by SCUBA (-30m) in the Tsitsikamma Marine Reserve, South Africa in May 1994. Portions (2g) of the ethyl acetate extracts of the freeze dried yellow and grey color variants were fractionated by flash chromatography on silica gel using a hexane/ethyl acetate solvent gradient. The fractions eluted with hexane/ethyl acetate (8:2) from the yellow color variant were combined and concentrated to give a crystalline residue. Normal-phase semi-prep. HPLC (hexane:ethyl acetate, 95:5) yielded 5α-pregna-1,20-dien-3-one (**1**, 80mg) together with a fraction containing a diterpene compound. Further normal-phase HPLC (hexane:ethyl acetate, 93:7) of this fraction gave tsitsixenicin A (**6**, 23mg)

Two fractions, eluted with hexane/ethyl acetate (8:2), from the flash chromatography of the grey color variant were concentrated separately and after further chromatography on normal-phase (hexane:ethyl acetate, 85:15) and reverse-phase (acetonitrile:water, 7:3) HPLC columns gave 16ß-5α-pregna-1,20-dien-3-one 16-acetate (**2**, 7mg) and 3α,16ß-dihydroxy-5α-pregna-1,20-diene 3,16-diacetate (**3**, 5mg). Several chromatography fractions containing diterpene compounds were isolated concurrently. Normal-phase (hexane:ethyl acetate, 85:15) and reverse-phase (acetonitrile:water, 73:27) HPLC of these fractions yielded tsitsixenicin A (**6**, 88mg) and tsitsixenicin B (**10**, 5mg), tsitsixenicin C (**11**, 6mg) and tsitsixenicin D (**12**, 8mg).

5α-pregna-1,20-dien-3-one (1): white needles, mp. 125-126°C, $[\alpha]_D^{17}$ = +45° (*c* 1.11, $CHCl_3$); IR ($CHCl_3$) 2943, 2873, 1674, 1448, 1274, 1213, 918 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.13 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 5.83 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-2), 5.74 (td, 1H, J = 16.5, 10.8, 8.8 Hz, H-20), 4.95 (m, 2H, H-21), 2.35 (dd, 1H, J = 17.7, 14.1 Hz, H-4), 2.20 (dd, 1H, J = 17.7, 3.6 Hz, H-4), 1.95 (m, 1H, H-17), 1.93 (m, 1H, H-5), 1.78 (m, 1H, H-6), 1.76 (m, 1H, H-15), 1.76 (m, 1H, H-12), 1.74 (m, 1H, H-7), 1.66 (m, 1H, H-16), 1.55 (m, 1H, H-6), 1.45 (m, 1H, H-8), 1.42 (m, 2H, H-11), 1.42 (m, 1H, H-15), 1.18 (m, 1H, H-16), 1.08 (m, 1H, H-14), 1.07 (m, 1H, H-12), 1.00 (s, 3H, H-19), 0.99 (m, 1H, H-7), 0.97 (m, 1H, H-9), 0.61 (s, 3H, H-18); EIMS (70eV), *m/z* (int, %), M^+ 298 (11), 283 (29), 134 (36), 122 (100), 121 (41), 95 (42), 79 (52), 67 (35)

Tsitsixenicin A (6): yellow oil; $[\alpha]_D^{17}$ = -64° (*c* 0.9, $CHCl_3$); IR ($CHCl_3$) 3090, 3038, 1960, 1821, 1735, 1482, 1230, 1039 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.52 (d, 1H, $J_{3,4a}$ = 1.9 Hz, H-3), 5.75 (d, 1H, $J_{1,11a}$ = 3.6 Hz, H-1), 5.37 (dd, 1H, J = 9.5, 8.0 Hz, H-8), 5.25 (t, 1H, $J_{12,13}$ = 7.6 Hz, H-12), 4.97 (t, 1H, J = 6.9 Hz, H-14), 4.91 (brs, 1H, H-19), 4.78 (brs, 1H, H-19'), 2.43 (m, 1H, H-13), 2.43 (m, 1H, H-9), 2.31 (m, 1H, H-10), 2.31 (m, 1H, H-13'), 2.29 (m, 1H, H-4a), 2.19 (m, 1H, H-6), 2.08 (m, 1H, H-5), 2.07 (m, 1H, H-6'), 2.07 (m, 1H, H-9'), 2.07 (m, 1H, H-10'), 2.07 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.96 (brs, 1H, H-11a), 1.67 (brs, 3H, H-18), 1.67 (brs, 3H, H-17), 1.64 (brs, 3H, H-16), 1.22 (m, 1H, H-5'); EIMS (70eV), *m/z* (int, %), No M^+, 231 (11), 83 (38), 69 (19), 55 (17), 43 (100).

16ß-hydroxy-5α-pregna-1,20-dien-3-one 16-acetate (2): colorless oil; $[\alpha]_D^{21}$ = +91° (*c* 0.58, $CHCl_3$); IR ($CHCl_3$) 2939, 1729, 1669, 1609, 1256 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.12 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 5.85 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-2), 5.77 (td, 1H, J=17.1, 10.5, 8.9 Hz, H-20), 5.11 (m, 1H, H-16), 5.08 (m, 2H, H-21), 2.36 (dd, 1H, J =

17.6, 14.1 Hz, H-4), 2.21 (dd, 2H, J = 17.7, 4.0 Hz, H-4), 2.15 (dd, 1H, $J_{17,20}$ = 7.7 Hz, $J_{16,17}$ = 4.1 Hz, H-17), 2.02 (s, 3H, OAc), 1.95 (m, 1H, H-5), 1.85 (m, 1H, H-11), 1.77 (m, 1H, H-15), 1.74 (m, 1H, H-12), 1.67 (m, 1H, H-7), 1.56 (m, 1H, H-11), 1.47 (m, 1H, H-8), 1.45 (m, 1H, H-6), 1.42 (m, 1H, H-15), 1.41 (m, 1H, H-6), 1.38 (m, 1H, H-14), 1.20 (m, 1H, H-12), 1.02 (m, 1H, H-9), 1.01 (s, 3H, H-19), 0.98 (m, 1H, H-7), 0.69 (s, 3H, H-18); EIMS (70eV) *m/z* (int, %), No M$^+$, 296 (41), 122 (73), 121 (45), 107 (45), 94 (73), 79 (76), 43 (100).

3α,16ß-dihydroxy-5α-pregna-1,20-diene 3,16-diacetate (3): colorless oil; $[\alpha]_D^{21}$ = +133.0° (*c* 0.53, $CHCl_3$); IR ($CHCl_3$) 3029, 2934, 1726, 1482, 1378, 1257, 1039 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.18 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 5.76 (td, 1H, J = 17.1, 10.4, 8.9 Hz, H-20), 5.60 (ddd, 1H, $J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 5.5 Hz, $J_{2,4}$ = 1.2 Hz, H-2), 5.15 (m, 1H, H-3), 5.08 (m, 1H, H-16), 5.04 (m, 2H, H-21), 2.13 (t, 1H, J = 7.7 Hz, H-17), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.85 (m, 1H, H-11), 1.81 (m, 1H, H-4), 1.76 (m, 1H, H-15), 1.71 (m, 1H, H-12), 1.68 (m, 1H, H-7), 1.58 (m, 1H, H-11), 1.58 (m, 1H, H-5), 1.53 (m, 1H, H-4), 1.40 (m, 1H, H-8), 1.37 (m, 1H, H-15), 1.37 (m, 1H, H-14), 1.37 (m, 2H, H-6), 1.14 (m, 1H, H-12), 1.02 (m, 1H, H-7), 0.99 (m, 1H, H-9), 0.81 (s, 3H, H-19), 0.66 (s, 3H, H-18); EIMS (70eV) *m/z* (int, %), No M$^+$, 120 (43), 105 (76), 95 (36), 94 (54), 91 (72), 43 (100).

Tsitsixenicin B (10): colorless oil; $[\alpha]_D^{21}$ = -38° (*c* 0.49, $CHCl_3$); IR ($CHCl_3$) 2960, 2934, 2864, 1726, 1239, 1074 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.51 (d, 1H, J = 1.3 Hz, H-3), 5.87 (d, 1H, J = 3.3 Hz, H-1), 5.24 (t, 1H, J = 7.6 Hz, H-12), 5.06 (brs, 1H, H-19), 4.95 (m, 1H, H-14), 4.93 (brs, 1H, H-19'), 2.98 (dd, 1H, J = 3.2, 10.8 Hz, H-8), 2.43 (m, 1H, H-11a), 2.41 (m, 1H, H-5), 2.41 (m, 1H, H-13), 2.38 (m, 1H, H-4a), 2.23 (m, 1H, H-5'), 2.23 (m, 1H, H-13'), 2.22 (m, 1H, H-9), 2.19 (m, 1H, H-6), 2.08 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.67 (s, 3H, H-17), 1.61 (s, 3H, H-16), 1.43 (m, 1H, H-9'), 1.35 (m, 2H, H-10), 1.31 (s, 3H, H-18), 1.19 (m, 1H, H-6'); EIMS (70eV), *m/z* (int, %), No M$^+$, 167 (31), 149 (100), 83 (25), 71 (22), 43(75).

Tsitsixenicin C (11): colorless oil; $[\alpha]_D^{21}$ = -138.9° (*c* 0.60, $CHCl_3$), IR ($CHCl_3$) 3620, 3029, 2934, 1735, 1613, 1221, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.25 (d, 1H, J = 2.0 Hz, H-3), 6.82 (d, 1H, $J_{13,14}$ = 15.8 Hz, H-14), 6.51 (d, 1H, $J_{13,14}$ = 15.8 Hz, H-13), 6.14 (d, 1H, J = 2.3 Hz, H-1), 5.59 (m, 1H, H-9), 5.40 (d, 1H, $J_{8,9}$ = 11.9 Hz, H-8), 5.14 (d, 2H, J = 7.0 Hz, H-19), 3.44 (m, 1H, H-4a), 3.17 (m, 1H, H-10), 2.89 (m, 1H, H-6), 2.63 (m, 1H, H-10'), 2.63 (m, 1H, H-11a), 2.06 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.73 (m, 1H, H-5), 1.61 (s, 3H, H-18), 1.57 (m, 1H, H-5'), 1.46 (m, 1H, H-6'), 1.40 (s, 3H, H-16), 1.39 (s, 3H, H-17); EIMS (70eV), *m/z* (int, %), No M$^+$, 91 (13), 85 (24), 83 (32), 69 (15), 55 (14), 43 (100).

Tsitsixenicin D (12): colorless oil; $[\alpha]_D^{21}$ = -126° (*c* 0.81, $CHCl_3$); IR ($CHCl_3$) 3029, 2934, 1735, 1613, 1230, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.26 (d, 1H, J = 2.0 Hz, H-3), 6.12 (d, 1H, J = 2.3 Hz, H-1), 5.57 (m, 1H, H-9), 5.41 (d, 1H, $J_{8,9}$ = 11.8 Hz, H-8), 5.30 (t, 1H, J = 7.0 Hz, H-14), 5.12 (d, 2H, J = 8.3 Hz, H-19), 3.32 (m, 1H, H-4a), 3.25 (t, 2H, J = 5.6 Hz, H-13), 3.16 (m, 1H, H-10), 2.84 (brt, 1H, J = 12.2 Hz, H-6), 2.65 (m, 1H, H-10'), 2.60 (d, 1H, J = 12.3 Hz, H-11a), 2.04 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.74 (d, 3H, J = 1.0 Hz, H-16), 1.69 (m, 1H, H-5), 1.64 (s, 3H, H-17), 1.59 (s, 3H, H-18), 1.55 (m, 1H, H-5'), 1.43 (m, 1H, H-6'); EIMS (70eV), *m/z* (int, %), No M$^+$, 245 (19), 135 (14), 85 (22), 83 (27), 69 (22), 55 (14), 43 (100).

Saponification of 16ß-hydroxy-5α-pregna-1,20-dien-3-one 16-acetate (2): A solution of **2** (3.4mg) in ethanol

(1ml) was refluxed (80°C, 1hr) with ethanolic KOH (0.24mmol). Water was added and the ethanol removed under reduced pressure. The aqueous solution was acidified, extracted with ether, dried over anhyd. Na_2SO_4, filtered and concentrated to yield 16ß-hydroxy-5α-pregna-1,20-dien-3-one (**4**) as a yellow oil (3.0mg); $[\alpha]_D^{17}$ = +12° (*c* 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.13 (d, 1H, J = 10.2 Hz, H-1), 5.85 (d, 1H, J = 10.7 Hz, H-2), 5.81 (m, 1H, H-20), 5.14 (m, 2H, H-21), 4.22 (m, 1H, H-16), 2.36 (dd, 1H, J = 17.6, 14.1 Hz, H-4), 2.22 (dd, 1H, J = 18.3, 4.2 Hz, H-4), 1.92 (m, 1H, H-5), 1.00 (s, 3H, H-19), 0.68 (s, 3H, H-18).

Reduction of 3α,16ß-dihydroxy-5α-pregna-1,20-diene 3,16-diacetate (3): LAH (8mg) was added to a stirred solution of **3** (3.5mg) in dry ether (2ml). The solution was cooled in ice and 5% NaOH (2ml) added. The basic solution was extracted with ether, dried over anhyd. Na_2SO_4, filtered and concentrated to give 5α-pregna-1,20-dien-3α,16ß-diol (**5**) as a white solid (3.1mg); $[\alpha]_D^{17}$ = -63° (*c* 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.06 (d, 1H, J = 10.0 Hz, H-1), 5.81 (m, 1H, H-20), 5.65 (m, 1H, H-2), 5.12 (m, 2H, H-21), 4.22 (td, 1H, J = 8.8, 2.1 Hz, H-16), 4.08 (td, 1H, J = 4.5 Hz, H-3).

Methylation of tsitsixenicin A (6): A solution of tsitsixenicin A (33mg) in MeOH (4ml), was refluxed (1.5hr), concentrated and purified by normal-phase HPLC (hexane:ethyl acetate, 9.3:0.7) to give compound **8** as a colorless oil (17mg), $[\alpha]_D^{21}$ = -95° (*c* 0.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.78 (d, 1H, J = 2.3 Hz, H-1), 5.41 (dd, 1H, J = 11.2, 3.6 Hz, H-8), 5.39 (t, 1H, J = 7.3 Hz, H-12), 5.15 (t, 1H, J = 7.0 Hz, H-14), 5.06 (s, 1H, H-3), 3.37 (s, 3H, OMe), 1.96 (s, 3H, OAc); ^{13}C NMR ($CDCl_3$) δ 170.0 (s), 135.2 (s), 132.7 (s), 125.4 (d), 121.5 (d), 113.9 (t), 103.4 (d), 90.2 (d), 54.8 (q), 40.4 (t), 27.0 (t), 25.6 (t), 25.6 (q), 21.3 (q), 17.4 (q).

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References

1. Williams, G.C. *Annals of the South African Museum*. **1992**, *100*, 249.
2. Lin, Y.; Bewley, C.A.; Faulkner, D.J. *Tetrahedron*, **1993**, *49*, 7977.
3. Hooper, G. J.; Davies-Coleman, M.T. *Nat. Prod. Lett.*, **1994** (in press)
4. Personal communication with Dr G. Williams, California Academy of Sciences, San Francisco.
5. Blackman, A.J.; Heaton, A.; Skelton, B.W.; White, A.H. *Aust. J. Chem.*, **1985**, *38*, 565.
6. Higgs, M.D.; Faulkner D.J. *Steroids*, **1977**, *30*, 379.
7. Eggert, H.; VanAntwerp, C.L.; Bhacca, N.S.; Djerassi, C. *J. Org. Chem.*, **1976**, *41*, 71. Kobayashi, M.; Appa Rao, K.M.C.; Anjaneyulu, V. *J. Chem. Research(S)*, **1994**, 140.

8. Das, B.; Srinivas, K.V. *J. Nat. Prod.*, **1992**, *55*, 1310.
9. Vanderah, D.J.; Steudler, P.A.; Ciereszko, L.S.; Schmitz, F.J.; Ekstrand, J.D.; van der Helm, D. *J. Am. Chem. Soc.*, **1977**, *99*, 5780. Groweiss, A.; Kashman, Y. *Tetrahedron Lett.*, **1978**, 2205. Kashman, Y.; Groweiss, A. *Tetrahedron Lett.*, **1978**, 4833. Braekman, J.C.; Daloze, D.; Tursch, B.; Declerq, J.P.; Germain, G.; Van Meersche, M. *Bull. Soc. Chim. Belg.*, **1979**, *88*, 71. Bowden, B.F.; Coll, J.C.; Ditzel, E.; Mitchell, S.J.; Robinson, W.T. *Aust. J. Chem.*, **1982**, *35*, 997. Lelong, H.; Ahond, A.; Chiaroni, A.; Poupat, C.; Riche, C.; Potier, P.; Pusset, J.; Pusset, M.; Laboute, P.; Menou, J.L. *J. Nat. Prod.*, **1987**, *50*, 207. Almourabit, A.; Ahond, A.; Chiaroni, A.; Poupat, C.; Riche, C.; Potier, P.; Laboute, P.; Menou, J.L. *J. Nat. Prod.* **1988**, *51*, 282. Green D., Carmely S., Benayahu, Y.; Kashman, Y. *Tetrahedron Lett.*, **1988**, 1605. Koning, G.M.; Coll, J.C.; Bowden, B.F.; Gulbis, J.M.; Mackay, M.F.; La Barre, S.C.; Laurent, D. *J. Nat. Prod.*, **1989**, *52*, 294. Almourabit, A.; Gillet, B.; Ahond, A.; Beloeil, J.C.; Potier, P. *J. Nat. Prod.*, **1989**, *52*, 1080. Almourabit, A.; Ahond, A.; Poupat, C.; Potier, P. *J. Nat. Prod.*, **1990**, *53*, 894.
10. Coval, S.J.; Scheuer, P.J.; Matsumuto, G.K.; Clardy, J. *Tetrahedron*, **1984**, *40*, 3823.
11. Ochi, M.; Kataoka, K.; Tatsukawa, A.; Kotsuki, H.; Shibata, K. *Heterocycles*, **1994**, *38*, 151.
12. Kashman, Y.; Groweiss, A. *J. Org. Chem.*, **1980**, *45*, 3814.
13. Tanaka, J.; Ogawa, N.; Liang, J.; Higa, Tatsuo, deNys, R.; Bowden, B.F., Carroll, A. R.; Coll, J. C.; Bernardinelli, G.; Jefford, C.W. *Tetrahedron*, **1994**, *50*, 9989.
14. Hokama, S.; Tanaka, J.; Higa, T.; Fusetani, N.; Asano, M.; Matsunaga, S.; Hashimoto, K. *Chemistry Lett.*, **1988**, 855.
15. Coll, J.C. *Chemical Reviews*, **1992**, *92*, 613.
16. Personal communication with Dr John Langlands, Inflazyme Pharmaceuticals, Vancouver, Canada.

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