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A PREGNANE ESTER TETRAGLYCOSIDE FROM *DREGEA LANCEOLATA*

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Key Word Index—*Dregea lanceolata*; Asclepiadaceae; dregenin; dregealin; steroid; pregnane ester; tetraglycoside.

Abstract—A new pregnane ester tetraglycoside designated as dregealin of a new genin named as dregenin was isolated from the dried roots of *Dregea lanceolata*. Chemical and spectroscopic evidences were consistent with the structure 11-*O*-acetyl-marsdenin-3-*O*-[*O*-α-L-diginopyranosyl-(1→4)-*O*-α-L-diginopyranosyl-(1→4)-*O*-β-D-cymaropyranosyl-(1→4)]-β-D-oleandropyranoside for dregealin.

INTRODUCTION

In several species of the Asclepiadaceae, cardenolides have been reported to be present together with pregnane derivatives [1]. In our continuing search for new compounds from *Dregea lanceolata*, a mixture of glycosides of 2-deoxy sugars was extracted, which by column chromatography over silica gel afforded a new crystalline pregnane ester tetraglycoside named dregealin (**1**) of a new genin, dregenin (**3**).

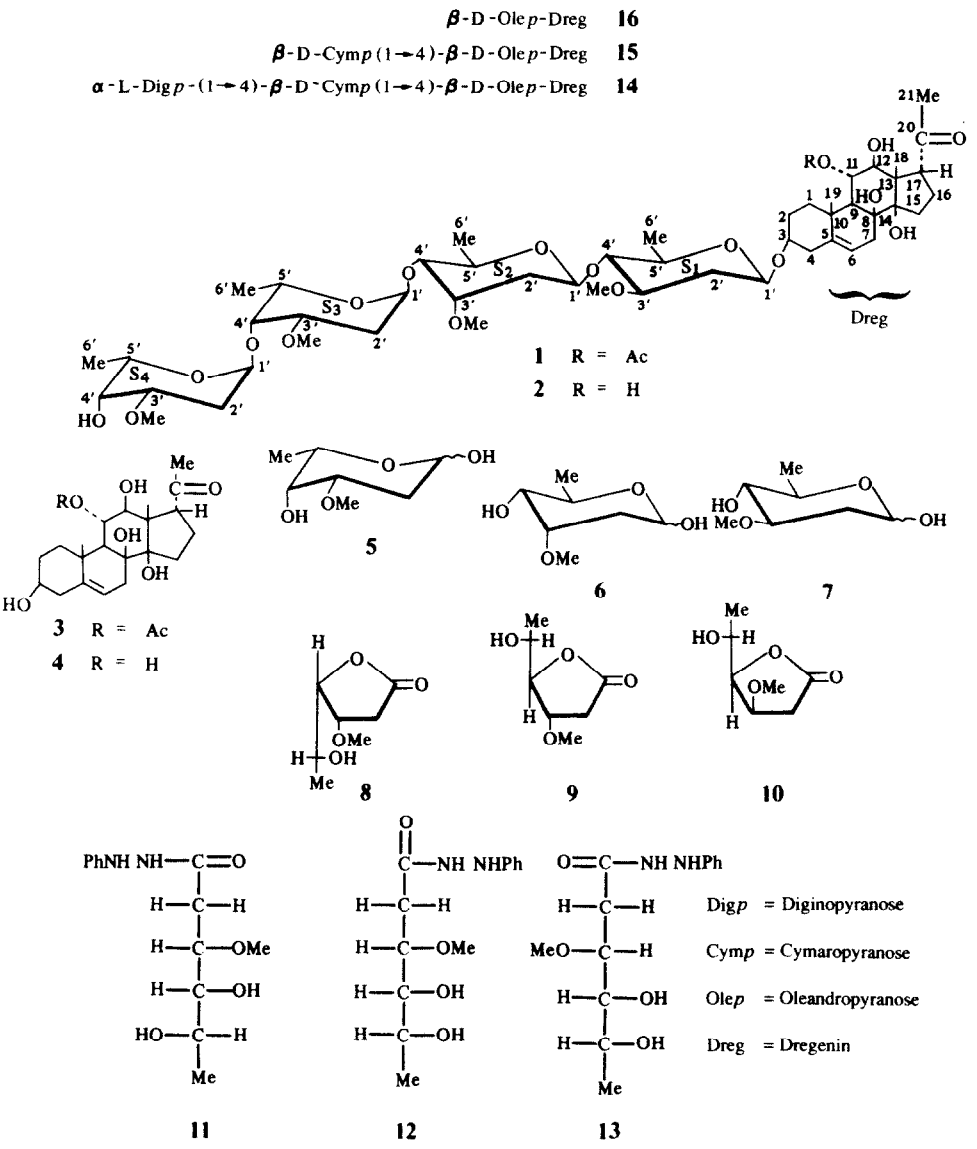
RESULTS AND DISCUSSION

Dregealin (**1**) mp 115°, $[\alpha]_D$ +28.57°, $C_{51}H_{82}O_{19}$ responded positively to the Liebermann–Burchardt test [2], xanthydrol [3, 4] and Keller–Kiliani [5] reactions, indicating it to be a steroidal glycoside of 2-deoxy sugars. In the IR spectrum of **1** the absorption maxima at 3400, 1740, 1366 and 770 cm^{-1} were assigned to hydroxyl, carbonyl, methyl deformations of a methyl ketone and trisubstituted double bond, respectively. The presence of a carbonyl group was further indicated by the ability of this compound to undergo facile reduction with sodium borohydride and its nature as a methyl ketone was established by a positive sodium nitroprusside test [6]. It underwent alkaline hydrolysis by the Zemplén method yielding a more polar product **2**, which indicated the presence of ester group(s) in **1**. In the ^1H NMR spectrum of **1**, the presence of four anomeric protons at δ 4.84 (1H), 4.80 (2H) and 4.78 (1H), together with four secondary methyl group doublets ($J=6$ Hz) of three protons each at δ 1.36, 1.28, 1.26 and 1.20, besides the characteristic methylene signals in the region δ 2.38–2.28 (4H) and 1.90–1.80 (4H) for equatorial and axial protons, respectively, provided evidence that **1** is a tetraglycoside possessing four 2,6-dideoxy hexosyl units. The ^1H NMR spectrum also contained a singlet of three protons at δ 1.96 for an acetyl group presumably present in the aglycone moiety of **1**.

To identify the aglycone moiety and sugars of **1**, it was hydrolysed by mild acid (0.025 M H_2SO_4) [7], affording a crystalline genin **3**, mp 162°, $[\alpha]_D$ +20° and a mixture of three sugars. The separated sugars **5**–**7** displayed characteristic colour tests of 2-deoxy sugars and were identified as L-diginose [8] (2,6-dideoxy-3-*O*-methyl-L-lyxohexose) (**5**), D-cymarose [9] (2,6-dideoxy-3-*O*-methyl-D-*ribo*-hexose) (**6**) and D-oleandrose [10] (2,6-dideoxy-3-*O*-methyl-D-arabinohexose) (**7**) (PC, $[\alpha]_D$). For further characterization compounds **5**–**7** were oxidized with bromine water to their lactones, **8**, **9** and **10**, respectively, which on treatment with phenylhydrazine yielded known crystalline derivatives, i.e. L-diginonic acid phenylhydrazide (**11**), D-cymaronic acid phenylhydrazide (**12**) and D-oleandronic acid phenylhydrazide (**13**), respectively. On the basis of the above results, compound **1** was characterized as a tetraglycoside containing L-diginose, D-cymarose and D-oleandrose moieties.

Genin, **3**, obtained on methanolysis by the Zemplén method [11, 12], afforded a crystalline product **4**, mp 262°, $[\alpha]_D$ −10°, confirming the presence of the ester group in **3**. From the melting point and specific rotation comparison, compound **4** was identified as 17α-marsdenin (3β,8β,11α,12β,14β-pentahydroxy-pregn-5-ene-20-one) [13]. The inertness of **1** and **3** to sodium metaperiodate, in contrast to their deacetylated products **2** and **4**, which reacted with this reagent, revealed that one of the hydroxyl groups in the vicinal diol arrangement was acetylated which could be at C-11 or C-12 of the genin. The location of the acetyl group at C-11 was confirmed with the help of the ^1H NMR spectrum which showed a low field one proton triplet at δ 5.35 ($J=8$ Hz) attributable to a C-11 methine proton and the higher field one proton doublet at δ 4.57 ($J=8$ Hz) assigned to a methine proton at C-12 bearing a free hydroxyl group. The genin **3** was thus identified as 11-*O*-acetyl marsdenin, reported here for the first time and named as dregenin (**3**).

More direct chemical support for compound **1** being a tetraglycoside of diginose, cymarose and oleandrose and determination of the sequence of the sugar units came from the results of its very mild acid (0.005 M H_2SO_4) hydrolysis. After four days, the reaction mixture exhibited two new spots (PC and TLC), one was found identical with diginose (**5**) and the other was presumed to be triglycoside **14**, leading to the conclusion that diginose was the terminal sugar. After eight days, a new spot, presumably the diglycoside **15**, appeared with the same spot of diginose sugar (**5**) indicating that the second sugar in the sequence was also diginose. After 12 days two



additional new spots appeared (PC and TLC); one was found identical in mobility with cymarose (**6**) and the other was presumed to be monoglycoside **16**, leading to the conclusion that cymarose was next to the diginose sugar units in the sequence. The hydrolysis was complete in 17 days exhibiting four spots identical in mobilities with diginose (**5**), cymarose (**6**), oleandrose (**7**) and the genin, dregenin (**3**). This led to the conclusion that oleandrose (**7**) was glycosidically linked to dregenin (**3**) at the C-3 hydroxyl group [16, 17] as compound **2** showed a positive reaction with sodium metaperiodate indicating the presence of a vicinal diol arrangement of the C-11 and C-12 hydroxyl groups of the genin moiety.

The EI mass spectrum of compound **1** did not exhibit a $[M]^+$, expected at *m/z* 999, but the highest mass ion peak at *m/z* 577 was attributed to a tetrasaccharide fragment, [M − genin fragment]$^+$, presumably originating from the tetraglycoside moiety. The other ion peaks at *m/z* 257 and 239 were attributed to [disaccharide fragment ion (289) − MeOH]$^+$ and [257 − H_2O]$^+$ ions, possibly originating from the tetrasaccharide moiety of the glycoside. The lower mass region contained the common fragment ion peaks for 2,6-dideoxy-monomethoxy hexose [14] at *m/z* 145, 113 and 95. The other prominent ion peak at *m/z* 422 was in agreement with the formula $C_{23}H_{34}O_7$ corresponding to [M − tetrasaccharide fragment]$^+$. The subsequent losses of an acetyl group and four water molecules from this ion giving ion peaks at *m/z* 362 [422 − HOAc]$^+$, 326 [362 − $2H_2O$]$^+$, 308 [362 − $3H_2O$]$^+$ and 290 [362 − $4H_2O$]$^+$ were in agreement with the presence of one acetyl group and four hydroxyl groups in its genin moiety.

The ^{1}H NMR ($CDCl_3$) spectrum at 300 MHz not only confirmed the tetraglycosidic nature of **1** but also helped in ascertaining the configuration of the glycosidic linkages. For convenience, the one oleandrose, one cymarose and two diginose units of **1** were designated as S_1, S_2, S_3 and S_4, respectively. The two double doublets appearing as broad doublets of one proton each at δ4.84 and 4.78 (J = 3 Hz) and two double doublets at δ4.80 (2H, J = 9.5

and 3 Hz) could be assigned to four anomeric protons of the four sugars. The small coupling constants (3 Hz) of two of these anomeric protons, typical of their equatorial configuration in a 2-deoxyhexopyranose moiety in the $^1C_4$ (L) conformation [15], were attributed to the S_3 and S_4 L-diginose units linked through α-L-(1→4) glycosidic bonds. The large coupling constant (9.5 Hz) of the other two anomeric protons typical of an axial configuration, attributed to the S_1 and S_2 sugar units, suggested D-oleandrose and D-cymarose moieties in $^4C_1$ (D) conformation [15] joined through β-D-glycosidic linkages. In addition to this the ^1H NMR spectrum of **1** also contained three singlets of four methoxy groups at δ3.66 (3H), 3.44 (6H) and 3.43 (3H) which could be assigned to the two diginose, one cymarose and one oleandrose units. The ^1H NMR spectrum also contained appropriate proton signals for the genin moiety consisting of 11-*O*-acetyl marsdenin (see Experimental).

On the basis of the above chemical and spectroscopic evidence the structure of dregealin (**1**) is 11-*O*-acetyl marsdenin-3-*O*- [*O*-α-L-diginopyranosyl-(1→4)-*O*-α-L-diginopyranosyl-(1→4)-*O*-β-D-cymaropyranosyl-(1→4)] β-D-oleandropyranoside.

EXPERIMENTAL

Mps: uncorr. Sugars were visualised with 50% aq. H_2SO_4 on TLC (silica gel G, BDH) and vanilline–$HClO_4$ reagent on PC. PC (Whatman no. 1) was performed using C_6H_5Me–BuOH (4:1) saturated with H_2O as developing solvent. Column chromatography was performed using silica gel (BDH, 60–120 mesh). ^1H NMR spectra: 300 and 90 MHz with TMS as int. standard.

Plant extraction. Shade-dried powdered roots of *Dregea lanceolata* (voucher No. 68836 deposited in the National Botanical Research Institute, Lucknow, India) were extracted and fractionated with solvents of different polarities, as reported earlier [16]. Repeated CC of the C_6H_6 extract (350.25 mg) over silica gel using $CHCl_3$–MeOH (24:1) as eluent afforded dregealin (60 mg).

Dregealin (**1**). Mp 115° (MeOH–Et_2O), $[\alpha]_D^{25}$ +28.57° (MeOH; *c* 0.175) (Found C 60.88; H 8.03; $C_{51}H_{82}O_{19}$ required C 61.32; H 8.21). It gave a positive Liebermann–Burchardt test and produced a pink colour in the xanthydrol and blue colour in the Keller–Kiliani reactions. It also gave a positive colour test with tetranitromethane and Na nitroprusside. It underwent reduction with $NaBH_4$ and alkaline hydrolysis with methanolic KOH. ^1H NMR (300 MHz): δ5.47–5.45 (1H, *m*, H-6), 5.35 (1H, *t*, *J* =8 Hz, H-11), 4.84 (1H, *dd*, *J* =3 and 1.5 Hz, H-1′ of diginose), 4.80 (2H, *dd*, *J* =9 and 3 Hz, H-1′ of cym and ole), 4.78 (1H, *dd*, *J* =3 and 1.5 Hz, H-1′ of diginose), 4.57 (1H, *d*, *J* =8 Hz, H-12), 3.90–3.83 (4H, *m*, H-5′ of S_1, S_2, S_3 and S_4), 3.60–3.48 (4H, *m*, H-3′ of S_1, S_2, S_3 and S_4), 3.66 (3H, *s*, OMe), 3.44 (6H, *s*, 2 × OMe), 3.43 (3H, *s*, OMe), 3.32–3.18 (4H, *m*, H-4′ of S_1, S_2, S_3 and S_4), 2.38–2.28 (4H, *m*, H-2′ eq. of S_1, S_2, S_3 and S_4), 2.17 (3H, *s*, 17-COMe), 1.96 (3H, *s*, C-11 OAc), 1.90–1.80 (4H, *m*, H-2′ ax. of S_1, S_2, S_3 and S_4), 1.36 (3H, *d*, *J* =6 Hz, 6′ Me), 1.28 (3H, *d*, *J* =6 Hz, 6′ Me), 1.26 (3H, *d*, *J* =6 Hz, 6′ Me), 1.20 (3H, *d*, *J* =6 Hz, 6′ Me), 1.10 (3H, *s*, 18-Me), 1.06 (3H, *s*, 19-Me). MS *m/z* (rel. int.): $[M]^+$ (not observed), 422 (7) $[M-\text{sugars}]^+$ ($C_{23}H_{34}O_7$), 362 (3) [422 −HOAc]$^+$ ($C_{21}H_{30}O_5$), 326 (2) $[362-2H_2O]^+$ ($C_{21}H_{26}O_3$), 308 (2) $[362-3H_2O]^+$ ($C_{21}H_{24}O_2$), 290 (2) $[362-4H_2O]^+$ ($C_{21}H_{22}O$), 284 (5) ($C_{14}H_{20}O_6$), 242 (3) ($C_{12}H_{18}O_5$), 224 (3) ($C_{12}H_{16}O_4$), 138 (5), ($C_9H_{14}O$), 120 (20) (C_9H_{12}), 179 (5) ($C_{11}H_{15}O_2$), 161 (6) ($C_{11}H_{13}O$), 222 (2) ($C_{13}H_{18}O_3$), 204 (3) ($C_{13}H_{16}O_2$), 140 (8) ($C_8H_{12}O_2$), 97 (21) (C_6H_9O). Sugar fragments: 577 (4) $[M-\text{genin fragment}]^+$ ($C_{28}H_{49}O_{12}$), 289 (2) $[577-\text{two sugars}]^+$ ($C_{14}H_{25}O_6$), 257 (3) $[289-MeOH]^+$ ($C_{13}H_{21}O_5$), 239 (2) $[257-H_2O]^+$ ($C_{13}H_{19}O_4$), 145 (5) ($C_7H_{13}O_3$), 113 (3) ($C_6H_9O_2$), 95 (15) (C_6H_7O).

Mild acid hydrolysis of dregealin (**1**). To a soln of **1** (15 mg) in 80% aq. dioxane (1 ml) was added 0.05 M H_2SO_4 (1 ml) and the soln was warmed for 30 min at 50°. Usual work-up as reported earlier [2], afforded a crystalline genin **3** (5 mg), mp 162° (MeOH–Me_2CO), $[\alpha]_D^{25}$ +20° (MeOH; *c* 0.1). ^1H NMR (90 MHz): δ 5.51–5.49 (1H, *m*, H-6), 5.38 (1H, *t*, H-11), 4.75 (1H, *d*, *J* =8 Hz, H-12), 4.49 (1H, *m*, H-3), 2.18 (3H, *s*, 17-COMe), 1.97 (3H, *s*, 11-OAc), 1.07 (3H, *s*, 18-Me), 1.00 (3H, *s*, 19-Me). MS *m/z* (rel. int.): $[M]^+$ (not observed), 422 (1), 362 (2), 344 (27), 326 (34), 308 (19), 290 (4), 284 (48), 242 (20), 224 (25), 222 (12), 204 (9), 179 (11), 161 (20), 140 (13), 138 (19), 120 (33) and 97 (3).

The aq. hydrolysate afforded the mixture of three sugars which were separated through CC($CHCl_3$–MeOH, 97:3) affording **5** (4 mg) $[\alpha]_D^{25}$ −63.2° (H_2O; *c* 0.1), **6** (2 mg) $[\alpha]_D^{25}$ +53.2° (H_2O; *c* 0.125) and **7** (1.6 mg) $[\alpha]_D^{25}$ −12.6° (H_2O; *c* 0.1). All these sugars gave positive colouration in xanthydrol, Keller–Kiliani and vanilline–perchloric acid reactions. The specific rotations, TLC and PC comparisons of **5**–**7** showed them to be identical to L-diginose (lit. [8] $[\alpha]_D$ −65°, H_2O), D-cymarose (lit. [9] $[\alpha]_D$ +55°, H_2O) and D-oleandrose (lit. [10], $[\alpha]_D$ −12.5°, H_2O), respectively.

$NaBH_4$ *reduction of* **1**. Compound **1** (3 mg) was dissolved in MeOH (1.2 ml) and $NaBH_4$ (3 mg) was added and the mixture kept for 2 hr at room temp. After usual work-up [17] the residue showed complete consumption of **1** and a spot of lower mobility (TLC, $CHCl_3$–MeOH, 19:1).

Hydrolysis of **1** *by the Zemplén method.* To a soln of **1** (2 mg) in abs. MeOH (0.5 ml) was added NaOMe (0.05 ml) and the mixture kept at room temp. After 30 min. it showed one spot of lower mobility for compound **2** (TLC, $CHCl_3$–MeOH, 47:3). When the reaction was complete (TLC), it was neutralized with IR 120H resin and filtered. MeOH was removed under red. pres. yielding the product **2** which responded positively to $NaIO_4$ reagent [17] indicating the presence of vicinal diol in **2**.

Hydrolysis of **3** *by the Zemplén method.* To a soln of **3** (3 mg) in abs. MeOH (1 ml) was added NaOMe (0.15 ml) and the mixture kept at room temp. When the reaction was complete (TLC), it was neutralized with IR 120H resin and filtered. MeOH was removed under red. pres. yielding a viscous product **4**, crystallized from MeOH–Et_2O, mp 262°, $[\alpha]_D^{25}$ −10° (MeOH *c* 0.306). This compound **4** also responded positively to $NaIO_4$ reagent indicating the presence of vicinal diol system in **4**. MS *m/z* (rel. int.): 380 (0.5), 242 (20), 224 (16), 206 (10), 163 (6), 138 (4), 120 (8) and 105 (18) [18].

Oxidation of **5** *with bromine water.* A soln of **5** (3.0 mg) in H_2O (0.6 ml) was mixed with Br_2 (12 μl) and shaken in a stoppered flask in the dark for 24 hr at room temp. Work-up of the reaction was carried out as usual [16] which yielded syrupy lactone **8** (2 mg). It gave violet colouration with NH_2OH–$FeCl_3$ spray reagent.

Oxidation of **6** *with bromine water.* A soln of **6** (1.5 mg) in H_2O (0.3 ml) was mixed with Br_2 (4 μl) as in the oxidation of **5** and afforded syrupy lactone **9** (1.2 mg) showing violet colouration with NH_2OH–$FeCl_3$ spray reagent.

Oxidation of **7** *with bromine water.* A soln of **7** (1.5 mg) in H_2O (0.3 ml) was mixed with Br_2 (4 μl) as in the oxidation of **5** affording syrupy lactone **10** (1.2 mg) showing a violet colouration with NH_2OH–$FeCl_3$ spray reagent.

L-*Diginonic acid phenylhydrazide* (**11**). A soln of lactone **8** (2 mg) in abs. EtOH (0.05 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml), and the mixture was heated for 30 min at 100°. The viscous mass was cooled and repeatedly

triturated with abs. Et_2O (to remove excess of phenylhydrazine), yielding L-diginonic acid phenylhydrazide (**11**) which crystallized from MeOH–Et_2O as needles, mp 131–133° (lit. [19] mp 132–135°).

D-*Cymaronic acid phenylhydrazide* (**12**). A soln of lactone **9** (1.2 mg) in abs. EtOH (0.04 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml) and heated as for **8**, afforded D-cymaronic acid phenylhydrazide (**12**) which crystallized from MeOH–Et_2O as needles, mp 154° (lit. [9] mp 155°).

D-*Oleandronic acid phenylhydrazide* (**13**). A soln of **10** (1.2 mg) in abs. EtOH (0.04 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml) and heated as for **8** to yield D-oleandronic acid phenylhydrazide (**13**), crystallized from MeOH–Et_2O as needles, mp 132° (lit. [8, 10] mp 135°).

Very mild acid hydrolysis of Dregealin (**1**). To a soln of **1** (10 mg) in 80% aq. dioxane (1.2 ml) was added 0.01 M H_2SO_4 (1.2 ml) and the soln was kept at room temp. After 4 days, TLC of the reaction mixture exhibited a spot due to diginose (**5**) (R_{dign} 1.00, taken as reference), and two more spots of mobilities (R_{dign} 2.20) and (R_{dign} 2.06) presumed to be triglycoside **14** and unhydrolysed starting material **1**, respectively. After 8 days, a new spot of mobility (R_{dign} 2.28) appeared which was presumed to be diglycoside **15**. After 12 days, two additional new spots appeared, one was found identical to cymarose (**6**) (R_{dign} 1.5) and the other was presumed to be monoglycoside **16** (R_{dign} 2.32). The hydrolysis was complete in 17 days, when two additional new spots, identical in mobilities with dregenin (**3**) (R_{dign} 3.5) and oleandrose (**7**) (R_{dign} 1.3) appeared. The reaction mixture was then worked-up followed by CC affording a crystalline dregenin (**3**), mp 162° (MeOH–Me_2CO), $[\alpha]_D^{25}$ +22° (MeOH; *c* 0.1) and three chromatographically pure reducing sugars as viscous syrups, viz. diginose (**5**), cymarose (**6**) and oleandrose (**7**), respectively.

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