# CULCITOSIDES $C_2$ AND $C_3$ FROM THE STARFISH Culcita novaeguineae

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Two steroid glycosides not previously described have been isolated from the digestive system of the starfish Culcite novaeguiniae, and these have been called culcitosides $C_2$ and $C_3$. With the aid of chemical and spectral methods, the chemical structure of $C_2$ has been established as 24ξ-methyl-5α-cholestane-3β,4β,6α,8,15β,16β,28-heptaol 28-O-[O-(2,4-di-O-methyl-β-D-xylopyranosyl)-(1 → 2)-α-L-arabinofuranoside, and that of $C_3$ as its 4-deoxy analogue.

We have previously [1] described the structure of culcitoside $C_1$ (I), a steroid glycoside from the tropical starfish Linckia guildingi and Culcita novaeguineae as 5α-cholestane-3β,4β,6α,8,15β,24ξ-hexaol 24-O-[O-(2,4-di-O-methyl-β-D-xylopyranosyl)-(1 → 2)-α-L-arabinofuranosidel].

Continuing an investigation of the steroid derivative from the digestive system of C. novaeguineae we have obtained two new glycosides, culcitosides $C_2$ (II) and $C_3$ (III).

The acid hydrolysis of (I-III) showed the identity of the compositions of their carbohydrate chains (arabinose and 2,4-di-O-methylxylose residues; TLC, PC), while the agreement of the values of the specific rotations of the mixtures of monosaccharides indicated that in culcitosidses $C_2$ (II) and $C_3$ (III) the arabinose belonged to the L series and the 2,4-di-O-methylxylose to the D series. In the products of the methylation of culcitosides $C_2$ (II) and $C_3$ (III) by Hakomori's method [2] followed by methanolysis and acetylation we found methyl 2,3,4-tri-O-methyl-α- and -β-xylopyranosides, which were identified by GLC and chromato-mass spectrometry, and this confirmed the terminal position of the 2,4-di-O-methylxylose residue.

The $^1H$ and $^{13}C$ NMR spectra for the carbohydrate chains of glycosides (II) and (III) coincided with the corresponding values for (I) (Tables 1 and 2). This definitely proved the complete identity of the carbohydrate chains of glycosides (I-III).

The signals of the $C_1$-$C_{14}$ carbon atoms (Table 2) and the characteristic signals of the $H_3$-$H_7$ protons determined from experiments on difference spin-decoupling (Table 1) in the spectra of culcitoside $C_2$ (II) agreed well with the corresponding values in the spectra of glycoside (I) [1]. Furthermore, in compound (II) the presence of an additional hydroxy group was observed, this being present at C-16 in ring D and having the β configuration. In actual fact, a comparison of the $^{13}C$ NMR spectra of (II) (Table 2) and of the model compound 5α-cholestane-3β,4β,6α,8,15β,16β,26-heptaol 6α-sulfate showed good agreement of the $C_{15}$, $C_{16}$, and $C_{17}$ signals [3]. Since the spin-spin coupling constants of the $H_{14}$, $H_{15}$, and $H_{16}$ atoms also show the 15α,16α-orientation of the protons, the 3β,4β,6α,8,15β, 16β arrangement of the hydroxy groups in the nucleus was ascribed to glycoside (II).

The positions and orientations of the hydroxy groups in the aglycon of cucitoside $C_3$ (III) were established by comparing its $^{13}C$ and $^1H$ spectra with those of glycosides (II) and (V) that we had isolated previously from the starfish Patiria pectinifera [4]. The chemical shifts of the $H_3$-$H_6$ protons and the corresponding spin-spin coupling constants in the $^1H$ NMR spectrum of compound (III) were close to those of the corresponding signals in the spectrum of substance (V) (Table 1), and the signals of the $H_{14}$, $H_{15}$, $H_{16}$, $H_{17}$, and $H_{20}$ protons coincides with the corresponding signals in the spectrum of culsitoside $C_2$. Consequently, in culcitoside $C_3$ (III) there was no hydroxy group at $C_4$, as in glycoside (V), and the positions of the other hydroxyls were the same as in culcitoside

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TABLE 1. $^1H$ NMR Spectra of Glycosides (II) and (III) ($C_5D_5N$, TMS = 0; δ, ppm; J*, Hz)

| Proton | II | III | Proton | II, III |
|---|---|---|---|---|
| H-3 | 4,00 m | 4,03 | | |
| H-4a | | 1,85 q (~12,2) | H-1′ | 5,60 d |
| H-4e | 5,26 t (2,5) | 3,13 dm (12,5) | H-2′ | 4,88 d |
| H-5 | 1,48 dd (2,1; 10,7) | 1,55 td (2,3; 10,6; 12,7) | H-3′ | 4,88 m |
| H-6 | 5,1 td (4,0; 10,5; 10,5) | 4,35 td (4,0; 10,5; 10,5) | H-4′ | 4,70 dd |
| H-7a | 1,90td (10,5; 12,5) | 1,85 dd (10,8; 12,3) | H-5′ | 4,40 dd |
| H-7e | 3,16 dd (4,5; 12,5) | 3,07 dd (4,6; 12,3) | H-5′ | 4,28 dd |
| H-14 | 1,14 d (5,8) | 1,14 d (5,5) | H-1″ | 4,98 d |
| H-15 | 4,68 dd (5,8; 7,0) | 4,69 dd (5,0; (7,1) | H-2″ | 3,40 dd |
| H-16 | 4,50 t (6,8) | 4,50 t (7,0) | H-3″ | 3,95 t |
| H-17 | 1,11dd (7,0; 11,1) | 1,10 dd (7,0; 11,0) | H-4″ | 3,53 m |
| H-20 | 2,30 m | 2,30 m | H-5″ | 3,35 dd |
| $CH_3$-18 | 1,637 s | 1,64 s | H-5″ | 4,23 t |
| $CH_3$-19 | 1,857 s | 1,40 | OMe | 3,53 s |
| $CH_3$-21 | 1,13 d | 1,15 d | OMe | 3,73 s |
| $CH_3$-26 | 0,94 d | 0,94 d | | |
| $CH_3$-27 | 0,97 d | 0,97 d | | |
| H-28 | 2,59 dd | 2,59 dd | | |
| H-28′ | 4,02 dd | 4,02 dd | | |

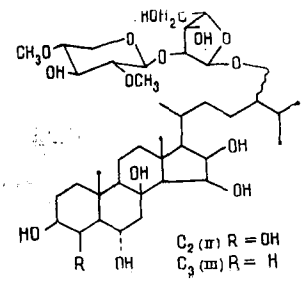
*The values of the splittings in multiplets. The spectra were recorded with an accuracy of 0.25 Hz/point.

TABLE 2. $^{13}C$ NMR Spectra of Glycosides (II) and (III) ($C_5D_5N$, δ, TMS = 0)

| Atom | II | III | Atom | II | III |
|---|---|---|---|---|---|
| C1 | 39,4 | 38,9 | C21 | 18,4 | 18,3 |
| C2 | 26,8 | 31,9 | C22 | 34,2 | 34,1 |
| C3 | 73,0 | 71,2 | C23 | 25,4 | 25,3 |
| C4 | 68,9 | 33,1 | C24 | 44,9 | 44,8 |
| C5 | 57,3 | 53,7 | C25 | 28,5 | 28,5 |
| C6 | 63,7 | 66,4 | C26 | 19,7 | 19,7 |
| C7 | 50,9 | 50,1 | C27 | 19,7 | 19,7 |
| C8 | 76,3 | 76,1 | C28 | 69,3 | 69,1 |
| C9 | 57,8 | 56,7 | C1′ | 107,7 | 107,6 |
| C10 | 37,6 | 37,4 | C2′ | 91,7 | 91,6 |
| C11 | 18,4 | 18,8 | C3′ | 77,5 | 77,4 |
| C12 | 42,8 | 42,7 | C4′ | 84,6 | 84,7 |
| C13 | 43,9 | 43,7 | C5′ | 62,5 | 62,4 |
| C14 | 60,7 | 60,2 | C1″ | 104,2 | 104,2 |
| C15 | 70,9 | 70,4 | C2″ | 84,8 | 84,7 |
| C16 | 71,8 | 71,7 | C3″ | 76,2 | 76,1 |
| C17 | 62,1 | 62,0 | C4″ | 80,5 | 80,4 |
| C18 | 18,0 | 17,9 | C5″ | 64,1 | 64,1 |
| C19 | 17,3 | 14,2 | OMe | 58,8 | 58,7 |
| C20 | 30,7 | 30,5 | OMe | 60,7 | 60,6 |

$C_2$ (II). A confirmation of this was the good agreement of the $C_7$, $C_8$, $C_{10-18}$, and $C_{20-28}$ signals in the $^{13}C$ NMR spectra of glycosides (II) and (III) (Table 2). We ascribed to culcitoside $C_3$ the 3β,6α,8,15β,16β arrangement of the hydroxy groups in the steroid nucleus.

It was shown by high-temperature catalytic hydrogenation (Pd, 330°C) followed by the identification (GLC and GLC-MS) of 24-methylcholestane in the hydrogenation products that both glycosides had a C-28 steroid skeleton.



The $^1H$ NMR spectra of glycosides (II) and (III) lacked the weak-field multiplet signal of $H_{24}$ that is characteristic for 24-O-glycosylated polyhydroxysteroids from starfish [5]. In place of this there were two one-proton doublets of doublets at 2.59 ppm (Table 1). Recording of the Overhauser effect on the irradiation of the anomeric proton of the arabinofurosyl residue (H-1') at 5.60 ppm in the $^1H$ NMR spectrum of glycoside (III) showed an enhancement of the signals at 2.59 and 4.02 ppm which indicated the attachment of the carbohydrate chain to a $CH_2$ group.

In additions to the signals of the $C_{11}$-$C_{19}$ carbon atoms already revealed, in the $^{13}C$ NMR spectra of both culcitosides there were another nine signals relating to the side chain: 30.5 (d), 18.3 (q), 34.1 (t), 25.3 (t), 44.8 (d), 28.5 (d), 19.7 (q), and 69.3 (t) ppm. These signals were assigned to the $C_{20}$-$C_{28}$ carbon atoms after a comparison of the values of their chemical shifts with the values for the corresponding carbon atoms in the spectrum of 24-ethylcholesterol [6].

The chemical shifts of the carbon atoms of the two C-24 epimers differed only slightly, and the mean values were used for comparison: (ppm) 36.0 ($C_{20}$), 18.7 ($C_{21}$), 33.7 ($C_{22}$), 26.2 ($C_{23}$), 45.7 ($C_{24}$), 28.9 ($C_{25}$), 19.2 ($C_{26}$), 19.2 ($C_{27}$), 22.9 ($C_{28}$).The values of the chemical shifts of the corresponding carbon atoms in the side chains of glycosides (II) and (III) agreed well with the models (Table 2), apart from the value for $C_{20}$, which was shifted upfield (by 5.3 and 5.5 ppm) through interaction with the β-hydroxy group at $C_{16}$.

The value of the chemical shift of the $C_{28}$ carbon atom (69.3 ppm) corresponded to the position of attachment of the carbohydrate chain.

On the basis of the facts given above, culcitoside $C_2$ was assigned structure (II) and culcitoside $C_3$ the structure of its 4-deoxyanalog (III). No glycerosides with such a steroid skeleton have previously been described in starfish.

## EXPERIMENTAL

All spectral characteristics and physical constants were determined under the conditions given in [7]. The $^{13}C$ and $^1H$ NMR spectra were taken on a Bruker WM-250 spectrometer. The Culcita novaeguineae starfish were collected in the north-western littoral of the island of Madagascar in February-March 1983.

Isolation of the Acetates of Culcitosides $C_2$ and $C_3$. A mixture of culcitosides $C_2$ and $C_3$, not separable by column chromatography, was isolated from the starfish (175 g) by a method described previously [5]. Acetylation of the mixture with acetic anhydride in pyridine in the usual way led to the combined acetates. The combined glycoside acetates (0.23 g) were separated by column chromatography on silica gel in the hexane–ethyl acetate (100:100) system and then on Florisol (200-300 mesh) in the hexane–ethyl acetate (180:100) system. This gave 0.06 g of the acetate of glycoside (II) and 0.03 g of the acetate of glycoside (III).

The acetate of glycoside (II) formed a colorless amorphous substance, $[\alpha]_D^{20}$ −16.7° (c 1.74; ethanol).

The acetate of glycoside (III) formed a colorless amorphous susbtance $[\alpha]_D^{20}$ −10° (c 0.15; ethanol).

Culcitoside $C_2$ (II) – $C_{40}H_{70}O_{15}$, mp 226-228°C, $[\alpha]_D^{20}$ −18° (c 0.1; ethanol) was isolated by the saponification of the acetate by the usual method. After purification on Polychrome and on Florisil in the chloroform–ethanol–water (300:90:water to saturation) system, 40.3 mg (0.023% on the ethanolic extract) was obtained.

Culcitoside $C_3$ (III) – $C_{40}H_{70}O_{14}$, mp 228-230°C, $[\alpha]_D^{20}$ −19.1° (c 0.17; ethanol. This was obtained by saponifying the acetate and subjecting the product to column chromatography. Yield 0.011% on the dry ethanolic extract.

Culcitosides $C_2$ and $C_3$ were methylated by Hakomori's method [2]. Methanolysis of the methylation products, acetylation, and identification of the completely methylated α- and β-xylopyranosides was carried out as described in [7].

Acid Hydrolysis. A mixture of 30 mg of a glycoside and 2 N HCl was heated at 85-95°C for 2 h. The monosaccharides were separated preparatively on Whatman 3 MM paper in the butanol–pyridine–water (10:3:3) system and were analyzed by TLC on silica gel impregnated

with 0.2 M sodium dihydrogen phosphate in the butanol–acetone–water (4:5:1) system and by GLC-mass spectrometry in the form of the corresponding aldonitrile peracetates. L-arabinose and 2,4-di-O-methyl-D-xylose were identified.

High-Temperature Hydrogenation of Culcitosides $C_2$ and $C_3$. Hydrogenation was performed with a mixture of 3 mg of a glycoside and 300 mg of catalyst [5% $Pd/CaCO_3$] as described in [8]. 24-Methylcholesterol was hydrogenated similarly. 24-Methylcholestane was identified in the reaction products by GLC and GLC-MS.

## SUMMARY

Two new steroid glycosides have been isolated from the starfish Culcita novaeguineae and characterized: 24ξ-methyl-5α-cholestane-3β,4β,6α,8,15β,16β, 28-heptaol 28-O-[O-(2,4-di-O-methyl-β-D-xylopyranosyl)-(1 → 2)-α-L-arabinopyranoside] – culcitoside $C_2$ – and its 4-deoxy analogue – culcitoside $C_3$.

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# WITHASTEROIDS OF Physalis.

## VII. 14α-HYDROXYIXOCARPANOLIDE AND 24,25-EPOXYWITHANOLIDE D

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Two new withasteroid have been isolated from Physalis angulata L. – 14α-hydroxyixocarpanolide and 24,25-epoxywithanolide D. 14α-hydroxyixocarpanolide – $C_{28}H_{40}O_7$, mp 245-250°C (from methanol), $[\alpha]_D^{20}$+29 ± 2° (chloroform). 24,25-Epoxywithanolide D – $C_{28}H_{38}O_7$, mp 257-261°C (from methanol, $[\alpha]_D^{20}$ +23 ± 2° (chloroform). On the basis of its UV, IR, CD, mass, and $^1H$ and $^{13}C$ NMR spectra the structure of 5α,14α,20R-trihydroxy-1-oxo-6α,7α-epoxy-22R-witha-2-enolide is suggested for 14α-hydroxyixocarpanolide. On the basis of spectral characteristics and the preparation of 4β-acetoxy and 4-oxo derivatives of 24,25-epoxywithanolide D, the structure of 4β,20R-dihydroxy-1-oxo-5β,6β;24S,25S-diepoxy-22R-witha-2-enolide is proposed for it.

We have begun an investigation of the plant Physalis angulata L. (Solanacaeae) growing in the territory of Tashkent province. As a result of the chromatographic separation of an extract we have obtained several compounds belonging, according to their spectral characteristics, to the withasteroids.

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