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RESEARCH ON AFRICAN MEDICINAL PLANTS—II†

HYPOXOSIDE, A NEW GLYCOSIDE OF UNCOMMON STRUCTURE FROM *HYPOXIS OBTUSA* BUSCH

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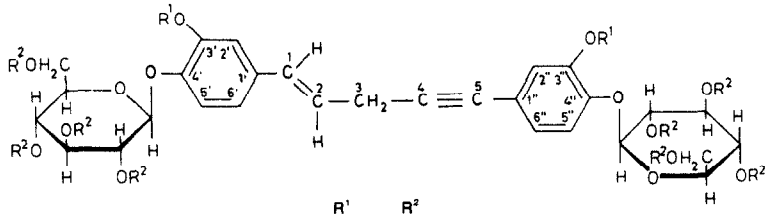
Abstract—A new glycoside, hypoxoside, was isolated from rhizome of *Hypoxis obtusa*, a plant used in Mozambique in native medicine for the treatment of urinary diseases. The structure **1** of a diglycoside of 1-(3′,4′-dihydroxyphenyl)-5-(3″,4″-dihydroxyphenyl)-1-penten-4-yne was attributed to hypoxoside on the basis of physico-chemical determinations and chemical behaviour.

Hypoxis obtusa Busch (Hypoxidaceae, fam. Amaryllidaceae) is a herbal plant, widespread in South East Africa, known in Mozambique under the local name of *Chirranga buharu*. Its bulbiform rhizome is dispensed directly by curanderos (healers) for preparing infusions for urinary diseases. Moreover the rhizome seems to be protected against mold and insect attack; in fact the inner part maintains yellow fresh flesh for a long period, but it becomes rapidly red and later black when cut and exposed to air.

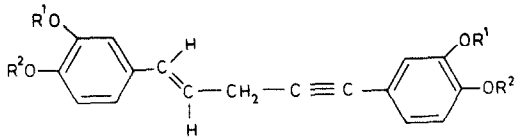
In order to isolate the active principles, the ground rhizome was extracted with MeOH and the extract residue (27%) was partitioned between H_2O and n-BuOH. Countercurrent distribution (CCD) between H_2O: AcOEt: n-BuOH gave substance **1** (3.7% of the rhizome), crystals from abs. EtOH, $C_{29}H_{34}O_{14}$, m.p. 149–151°, $[\alpha]_D^{20} = -73.5$ (MeOH). The substance, named by us hypoxoside, very soluble in water and methanol, gave on acetylation with Ac_2O and pyridine a crystalline decaacetate, **2**, $C_{29}H_{24}O_{14}(Ac)_{10}$, m.p. 127–129°. Substance **1** showed UV maxima (MeOH) at 258, 291, 298, 310 nm (log ϵ 3.46, 3.00, 3.00, 2.39) and underwent irreversible reaction with alkali. However, **1** gave a positive reaction for phenolic groups with ferric ferricyanide and on methylation with diazomethane it gave a crystalline dimethyl derivative, **3**, $C_{29}H_{32}O_{14}(Me)_2$, m.p. 157–159°, which by subsequent acetylation gave a crystalline octaacetate, **4**, $C_{29}H_{24}O_{14}(Me)_2$ $(Ac)_8$, m.p. 173–175°. In fact, the ^1H NMR spectrum of **4** (Varian XL 100) showed signals of eight acetyl groups (four at δ 2.04 and four at δ 2.06) and of two aromatic methoxy groups (δ 3.80 and 3.82). The signals at δ 3.34 (2H, d, J = 5 Hz), δ 6.15 (1H, dt, J = 5 and 16 Hz), δ 6.62 (1H, d, J = 16 Hz) were assignable to the sequence $-CH_2-CH=CH-$, with a *trans* relationship between the two olefinic protons on the basis of their large coupling constant. Furthermore, the signals of two equally substituted aromatic systems, formed by two *ortho* protons, δ 6.88 (dd, J = 2 and 8 Hz) and 7.07 (d, J = 8 Hz), of which the former showed an additional *meta* coupling with a hydrogen at δ 7.00 (d, J = 2 Hz), were present. Finally, in the same spectrum of **4**, signals ascribable to a glycidic moiety were evident. This accounts for the polarity of **1** and the formation of the octaacetate **4** from **3**. At this point, substance **1** was submitted to enzymatic hydrolysis by β-glucosidase (further hydrolyses were carried out with cellulase) after unprofitable attempts at acidic hydrolysis. After precipitation of the aglycone and its complete removal by extraction with AcOEt, D-glucose was identified as the sole monose in the aqueous solution and was confirmed through its β-pentaacetate[1] by comparison with an authentic specimen (IR, NMR, $[\alpha]$). Aglycone **5**, m.p. 154–156° (from AcOEt), $C_{17}H_{14}O_4$, M^+ at *m/e* 282 (base peak) gave with diazomethane an unstable oily tetramethyl derivative **6**, M^+ at *m/e* 338 (base peak), 4 OMe at δ 3.87, whereas with pyridine and Ac_2O, **5** also gave an oily unstable tetraacetyl derivative **7**, M^+ at *m/e* 450 (1), 4 Ac at δ 2.27. The chemical shifts of the methoxy groups of **6** as well as those of the acetyl groups of **7** accounted for four phenolic hydroxyls in **5**. Moreover, in **7** a weak IR band in $CDCl_3$ at 2220 cm^{-1} indicated the presence of an acetylenic bond, completely substituted, because of (i) the absence of IR bands in 3200–3300 cm^{-1} region, (ii) the absence of ^1H NMR signals in δ 2.3–3.0 region, (iii) the presence in the ^{13}C NMR spectra of **2–7** and **10** of two signals between 77 and 87 ppm (singlets in the SFORD spectra).

In agreement with the above-reported data, aglycone **5** took up 3 hydrogen moles/mole by hydrogenation with $Pt/BaSO_4$ and gave an 1-5-diphenylpentane derivative **8**, $C_{17}H_{20}O_4$, m.p. 126–127.5° (from AcOEt and n-hexane), M^+ at *m/e* 288 (54), wherein four hydroxy groups, symmetrically arranged, are two to two *ortho* as suggested from a bathochromic shift of 6 nm observed in UV spectrum on addition of AcONa and boric acid.[2] Based on the pattern of aromatic substitution for the examined

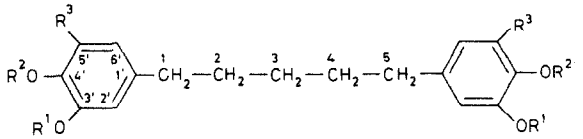
†Part I. J. U. Oguakwa, M. Patamia, C. Galeffi, I. Messana and M. Nicoletti, *Planta Med.* **41**, 410 (1981).



	R¹	R²
1:	H	H
2:	Ac	Ac
3:	Me	H
4:	Me	Ac



	R¹	R²
5:	H	H
6:	Me	Me
7:	Ac	Ac
9:	Me	H
10:	Me	Ac



	R¹	R²	R³
8:	H	H	H
11:	Me	β-D-glucopyranosyl	H
12:	Me	H	H
13:	Me	H	D

compounds (two *ortho* hydrogens, one of which has further *meta* coupling with another hydrogen) the 3', 4' positions for the hydroxy groups could be assigned unambiguously and therefore we propose the formula **8** for the hydrogenated aglycone.

The similarity between the UV spectrum of **3** (see experimental) and that of 1-5-diphenyl-1-penten-4-yne, obtained by synthesis,[3] (λ_{max} (EtOH) 239, 254, 293, 305 nm (log ϵ 4.40, 4.49, 3.25, 2.65)), indicated the same sequence of chromophores and therefore formula **5** for the aglycone of hypoxoside **1**.

In order to establish the positions of the two glucose moieties, dimethylhypoxoside **3** was hydrolyzed with cellulase: the oily product obtained **9**, M^+ at *m/e* 310 (base peak), altered quickly as well as its diacetyl derivative **10**, also oily, M^+ at *m/e* 394 (14), 2 OMe at δ 3.82 and 3.84, and 2 Ac at δ 2.28.

Therefore, before hydrolysis, dimethylhypoxoside, **3**, was hydrogenated with $Pt/BaSO_4$ and the resulting product **11**, $C_{31}H_{44}O_{14}$, m.p. 118–120° (from EtOH and AcOEt) was hydrolyzed with cellulase. The aglycone **12** crystallized from n-hexane and AcOEt, m.p. 77.5–78°, $C_{19}H_{24}O_4$, M^+ at *m/e* 316 (44). In the ^1H NMR spectrum of **12**, the perfect signal identity of two aromatic rings and of OMe groups (also with high resolution apparatus as Varian XL 100) indicated identity for the positions of the two hydroxyls in the aromatic rings and therefore for the positions of the glucose moieties in **11**. Through exchange with alkaline deuterium oxide, according to the procedure described by Kirby and Ogunkoya,[4] compound **12** gave compound **13** having two additional mass units, M^+ at *m/e* 318 (46). In its ^1H NMR spectrum the signal at δ 7.01 (d, J = 8 Hz) of the starting compound had disappeared, whereas the signals of protons H-2' and H-6' (δ 6.81) were scarcely split. This demonstrated that the free hydroxy groups in **12** were in 4' positions in each ring, because deuterium exchange had involved the hydrogen (one of each ring, *ortho* to OH group), which showed *ortho* and not *meta* coupling.

Therefore, in **11** as well as in **3** and in hypoxoside, **1**, residues of glucose were found to be bonded in positions *para* to the chain juncture. A β-glycosidic linkage was established by easy hydrolysis with β-glucosidase, whereas the β-pyranosidic structure, not assignable due to the overlapping of the signals in the ^1H NMR spectra of glycosides **2–4**, was established by comparison of ^{13}C NMR signals (Table 1) with the corresponding phenyl β-glycopyranosides.[5]

The ^{13}C NMR data of **2–7**, **10** and **13** were reported in Table 1. The distinction between C(1') and C(1'') shifts in **2–7** and **10** were made on the basis of the shielding effect of the acetylenic bond. On account of the absence of *ortho, ortho'* disubstitution, the signals of the aromatic methoxy groups in methyl derivatives **3**, **4**, **6**, **10** and **13** are lower than 60 ppm.

For the two identical glucose moieties, the signals are identical except for the slight difference of the anomeric carbon atoms, owing to the different substitution-site. The perfect identity of signals in **13** of the two aromatic rings is in agreement with the identical substitution, moreover

Table 1. ^{13}C NMR Chemical shifts assignments[a]

Compound	2	3	4	5	6	7	10	13
Solvent	$CDCl_3$	D_2O	$CDCl_3$	acetone-d_6	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$
C(1)	129.8	132.5	130.5	129.9	130.9	129.9	130.7	31.7
C(2)	122.7	120.4	118.6	117.7	119.1	121.9	118.7	35.6
C(3)	24.5	n.o.	22.6	21.5	22.9	22.8	22.9	29.0
C(4)	86.5	87.4	85.9	83.6	85.2	87.2	86.4	35.6
C(5)	81.6	83.7	82.1	81.5	82.5	77.2	77.2	31.7
C(1')	132.7	133.5	133.5	128.8	130.1	135.9	139.6	134.6
C(1")	119.7	118.3	119.4	114.2	110.0	123.7	122.1	134.6
C(2'),C(2")	115.0,115.2	110.8,110.8	110.1,115.5	111.9,114.4	108.8,110.9	124.3,124.3[b]	110.0,115.4	111.0
C(3'),C(3")	139.7,140.1	146.2,146.9	145.2,145.7	143.7,143.8[b]	148.3,148.4[b]	141.8,141.9[c]	150.6,150.9[b]	146.2
C(4'),C(4")	147.5,148.1	149.1,149.4	149.8,150.3	144.0,144.5[b]	148.7,148.9[b]	141.6,141.7[c]	150.6,150.9[b]	143.4
C(5'),C(5")	124.0,124.8	116.2,116.8	119.2,119.6	114.4,117.4	114.3,122.3	123.0,125.1[b]	122.6,122.7	
C(6')	126.5	126.4	123.5	120.7	124.6	126.5	124.3[c]	120.7
C(6")	130.1	127.6	124.1	122.9	126.9	129.8	124.4[c]	
glucose								
C(1)	98.4, 98.5	101.4,101.5	100.2,100.4					
C(2)	71.6[b]	69.9	71.7[b]					
C(3)	72.6[b]	76.8[b]	72.3[b]					
C(4)	68.3	67.9	68.2					
C(5)	72.1[b]	73.7[b]	72.1[b]					
C(6)	61.9	61.3	61.7					
OMe		56.5	55.8		55.8		55.9	56.9
COMe	168.3,168.6		168.8,168.9			167.7,167.9	168.5,168.8	
"	169.1,169.2		169.6,170.0					
"	169.9,170.1							
COMe	20.3, 20.5		20.3, 20.7			20.5	20.6, 20.7	
"	20.6							

[a]Chemical shifts in ppm downfield from TMS; dioxane was used as internal standard in D_2O and acetone-d_6 solutions
[b,c]Signals may be interchanged in the same column
n.o. = not observed

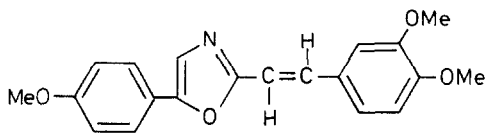
the disappearance of C(5') signal is due to the replacement of H with D.

The instability, particularly also in mild alkaline medium, of some derivatives of aglycone **5**, such as **6**, **7**, **9** and **10**, could be related to the reactivity of the methylene group already observed in analogous 1-en-4-yne systems.[3]

Hypoxoside **1** is the first type of natural product with a 1-en-4-yne structure. A C_6–C_2–C_3–C_6 system is rather uncommon in natural products from plants, although a biogenetic route from two phenylalanine units may be suggested for its formation.

This route has been proved for annuloline **14**, an oxazole isolated from *Lolium multiflorum* (Gramineae)[6] where a C_6–C_2 and C_3–C_6 system is present although separated by an oxygen and a nitrogen atom forming the oxazole ring.

Research is in progress in order to establish the biological activity of hypoxoside.



14

EXPERIMENTAL

A Craig Post apparatus (200 stages, 10:10 ml upper and lower phase) was used for countercurrent distribution (CCD). The separations, as well as the rate of reactions, were monitored by tlc analysis on silica gel F_{254}, if not otherwise reported. Solvent 1: n-BuOH : AcOH : H_2O 4:1:5 (upper phase); solvent 2: $CHCl_3$: AcOEt 1:1; substances were recovered from lower aqueous phase by repeated extractions with the upper phase. Spots were detected by short wave UV light. Mass spectra were obtained on a 9000 S spectrometer. ^1H NMR spectra were recorded with a Varian T 60, if not otherwise reported. ^{13}C NMR spectra were recorded with a Varian XL 100 (using $CDCl_3$ as solvent, if not otherwise reported, and TMS as reference). β-Glucosidase is β-glucosidase EG. 3.2.121 (Fluka). Cellulase is Cellulase EG.1.2.14 (Fluka).

Plant. Hypoxis obtusa is a herbal plant with leaves starting from the ground and bright yellow tepals. The rhizomes were purchased the first time in the market of Shipamanime, near the Maputo airport, by Dr. B. Bertuccioli (Rome) from a curandero who claimed its properties. Later Dr. T. Conforti could obtain further samples from the Instituto Nacional de Investigaçiones Agronomicas, where the plant was determined by Mr. L. Macuàcua. A sample of the plant is at the Istituto dell'Orto Botanico, Università di Roma under the number C-104.

Extraction. One four-month-old rhizome (214 g) was made to a mush and eluted with MeOH (21). The residue of the extract (58 g) was dissolved in water (200 ml) and the solution was extracted with AcOEt (3 × 150 ml, residue 1.6 g) and then with n-BuOH sat. with H_2O (4 × 150 ml). The residue of the pooled butanolic extracts amounted to 26 g.

Hypoxoside **1**. Part of the residue (5 g) was submitted to CCD (400 transfers) between H_2O:AcOEt:n-BuOH (10:8:2 v:v;v) (tlc, solvent 1). **1** (1.5 g, $K_r = 0.46$) crystallized from abs. EtOH by rubbing with glass stick, m.p. 149–151°; UV (MeOH) λ_{max}: 258, 291, 298, 310 nm (log ε 3.46, 3.00, 3.00, 2.39). The substance gave a positive reaction with potassium ferricyanide-ferric chloride $[\alpha]_D^{20} = -73.5$ ($c = 0.9$, MeOH) (Found: C, 56.82; H, 5.02. Calc. for $C_{29}H_{34}O_{14}$: C, 57.42; H, 5.65%).

Decaacetylhypoxoside **2**. Hypoxoside **1** (100 mg) was acetylated with a mixture of pyridine and Ac_2O (4 ml, 1:1). After a day, the reagents were evaporated under vacuum and the residue was crystallized from AcOEt and n-hexane, m.p. 127–129°, $[\alpha]_D^{20} = -44.4$ ($c = 0.9$, MeOH), ^1H NMR, δ: 2.06 (24 H, s, 8 Ac), 2.24 and 2.26 (6 H, 2 s, 2 phenolic Ac), 3.30 (2 H, d, J = 5 Hz, H_2-3), 4.20 (4 H, m, 2 CH_2OAc), 4.9–5.4 (10 H, monose methines), 6.06 (1 H, dt, J = 5 and 16 Hz, H-2), 6.58 (1 H, d, J = 16 Hz, H-1), 6.9–7.2 (6 H, aromatic)(Found: C, 57.47; H, 5.09. Calc. for $C_{49}H_{54}O_{24}$: C, 57.31; H, 5.30%).

Dimethylhypoxoside **3**. An ethereal solution (0.3 l) of CH_2N_2 (from 10 g of N-nitroso-N-methyl-4-toluensulfonamide) was added to a methanolic solution (0.8 l) of hypoxoside (1 g). After 7 days at 15° the starting substance was still present (tlc, solvent 1). The solvents were evaporated and the residue was purified by CCD between H_2O : AcOEt : n-BuOH 10:8:2 ($K_r = 0.28$†). Compound **3** (0.78 g), soluble in water, crystallized from abs. EtOH, m.p. 157–159°; UV (MeOH) λ_{max}: 260, 286, 297, 310 nm (log ε 3.43, 3.06, 3.02, 2.60); $[\alpha]_D^{20} = -66.3$ ($c = 0.7$, MeOH); MS, *m/e*(%): 634 (1), 619 (9), 312 (17), 310 (21), 60 (100) (Found: C, 58.25; H, 5.78. Calc. for $C_{31}H_{38}O_{14}$: C, 58.67; H, 6.04%).

Dimethyl-octaacetylhypoxoside **4**. The acetylation of **3** was carried out as for **2**. The product obtained crystallized from benzene and n-hexane, m.p. 173–175°, IR ($CHCl_3$), ν_{max}: 2150–2190 cm^{-1} broad; ^1H NMR (Varian XL 100), δ: 2.04 and 2.06 (24 H, 2s, 4 + 4 Ac), 3.34 (2 H, d, J = 5 Hz, H_2 – 3), 3.80 and 3.82 (6H, 2s, 2 OMe), 4.24 (4 H, m, 2 CH_2OAc), 4.9–5.5 (10 H, monose methines), 6.15 (1 H, dt, J = 5 and 16 Hz, H-2), 6.62 (1 H, d, J = 16 Hz, H-1), 6.88 (2 H, dd, J = 2 and 8 Hz, H-6' and 6''), 7.00 (2 H, d, J = 2 Hz, H-2' and 2''), 7.07 (2 H, d, J = 8 Hz, H-5' and 5'') (Found: C, 58.22; H, 5.39. Calc. for $C_{47}H_{54}O_{22}$: C, 58.14; H, 5.60%).

Hydrolysis of hypoxoside with β-glucosidase

Aglycone **5**. Phosphate buffer soln at pH 5.5 (40 ml) was added to an aqueous soln (120 ml) of **1** (500 mg) and of β-glucosidase (20 mg). The soln, covered by toluene and allowed to stand at 36°, precipated a white solid after 3 h. After a night, the hydrolysis was complete (tlc, solvent 1). Some drops of AcOH were added and then AcOEt was added to dissolve the solid and extract the aqueous phase. The residue of the organic phase was submitted to CCD (200 stages), solvents: H_2O : AcOEt : EtOH : cyclohexane 10:3:3:10. The aglycone **5** ($K_r = 0.42$, 197 mg), slight soluble in $CHCl_3$ was recrystallized from AcOEt, m.p. 154–156°; MS: M^+ at *m/e* 282 (base peak); ^1H NMR (MeOH-d_4) δ: 3.35 (2 H, d, J = 5 Hz, H_2-3 partially overlapped by solvent signals), 6.00 (1 H, dt, J = 5 and 16 Hz, H-2), 6.55 (1 H, d, J = 16 Hz, H-1), 6.6–7.0 (6 H, aromatic) (Found: C, 72.13; H, 4.97. Calc. for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00%).

Hydrolysis of hypoxoside with cellulase. Subsequent hydrolyses of **1** were carried out with cellulase Fluka (weight ratio 2:1) at 33° with acetate buffer at pH 4.5 following the previous procedure. After AcOEt extraction, the aqueous soln was extracted with n-BuOH and then percolated through a column of Dowex 50 W (H^+). The dried residue was submitted to column chromatography (cellulose, solvent: n-BuOH sat. with H_2O and with some drops of ammonia added) to purify the monose, which was identified as D-glucose by paper chromatography (Whatman 1, solvent 1, Tollens reagent) and through the corresponding β-pentaacetate[1] by comparison of IR and ^1H NMR spectra and rotatory power with an authentic specimen of β-D-pentaacetylglucose.

Tetramethylaglycone **6**. **5** dissolved in MeOH was methylated with an ethereal soln of CH_2N_2. After 3 days the solvents were evaporated and the residue was purified by CCD between H_2O : acetone : cyclohexane 8:12:14 ($K_r = 0.66$, tlc, solvent 1). The oily compound is very unstable. MS, *m/e* (%): 338 (M^+, $C_{21}H_{22}O_4$, 100), 323 (41), 308 (12), 307 (28); ^1H NMR, δ: 3.32 (2 H, d, J = 5 Hz, H_2-3), 3.87 (12 H, s, 4 OMe), 6.04 (1 H, dt, J = 5 and 16 Hz, H-2), 6.65 (1 H, d, J = 16 Hz, H-1), 6.7–7.1 (6 H, aromatic).

Tetraacetylaglycone **7**. **5** was acetylated as reported for **2**. The oily residue (one spot, tlc, solvent 2), resisted any attempt at crystallization. The compound is also very unstable. MS, *m/e* (%): 450 (M^+, $C_{25}H_{22}O_8$, 1), 408 (3), 366 (6), 324 (6), 282 (11); IR ($CHCl_3$) ν_{max}: 2220 cm^{-1} (weak); ^1H NMR, δ: 2.27 (12 H, s, 4 Ac), 3.34 (2 H, d, J = 5 Hz, H_2-3), 6.18 (1 H, dt, J = 5 and 16 Hz, H-2), 6.66 (1 H, d, J = 16 Hz, H-1), 7.1–7.3 (6 H, aromatic).

†It is remarkable that **3** has K_r and R_f values less than **1**.

Hexahydroaglycone **8**. By hydrogenation in presence of $Pt/BaSO_4$ 5% (120 mg) compound **5** (150 mg), dissolved in 90% MeOH (50 ml), absorbed within 1 h 3 H_2 moles/mole. After one night, the catalyst was removed by filtration and the dried residue of the soln was crystallized from AcOEt and n-hexane, m.p. 126–127.5°, UV (MeOH) λ_{max}: 224, 283 nm (log ϵ: 3.60, 3.38), + AcONa and H_3BO_3: 289 (3.55); MS, *m/e* (%): 288 (M^+, 54), 137 (5), 123 (100); ^1H NMR (acetone-d_6), δ: 1.2–1.9 (6 H, H_2-2, H_2-3, H_2-4), 2.55 (4 H, approx. t, H_2-1, H_2-5), 6.53(2 H, dd, J = 2 and 8 Hz, 2 H-6'), 6.70 (2 H, d, J = 2 Hz, 2 H-2'), 6.83 (2 H, d, J = 8 Hz, 2 H-5'), 7.60 (4 H, exchangeable with D_2O, 4 OH) (Found: C, 70.77; H, 6.86. Calc. for $C_{17}H_{20}O_4$: C, 70.81; H, 6.99%).

Hydrolysis of **3**

Compound **9**. Hydrolysis of **3** with cellulase was carried out according to the procedure reported for **1**. The residue of AcOEt (one spot, tlc, solvent 2, detection with potassium ferricyanide-ferric chloride) in spite of the purification by CCD (H_2O: acetone: cyclohexane 4:6:7, K_r = 0.20) remained oily. The compound is also very unstable. M^+, $C_{19}H_{18}O_4$, at *m/e* 310 (base peak); ^1H NMR, δ: 3.30 (2 H, d, J = 5 Hz, H_2-3), 3.85 (6 H, s, 2 OMe), 5.80 (2 H, exchangeable with D_2O, 2 OH), 6.08 (1 H, dt, J = 5 and 16 Hz, H-2), 6.67 (1 H, d, J = 16 Hz, H-1), 6.8–6.9 (6 H, aromatic).

Compound **10**. **9** was acetylated according to the routine procedure. The oily residue (one spot, tlc, solvent 2) in spite of the purification by CCD (H_2O: EtOH: acetone: n-hexane 10:10:5:20, K_r = 0.21) resisted any attempt at crystallization. MS, *m/e* (%): 394 (M^+, $C_{23}H_{22}O_6$, 14), 352 (44), 310 (100); ^1H NMR, δ: 2.28 (6 H, s, 2 Ac), 3.35 (2 H, d, J = 5 Hz, H_2-3), 3.82 and 3.84 (6 H, 2 s, 2 OMe), 6.20 (1 H, dt, J = 5 and 16 Hz, H-2), 6.67 (1 H, d, J = 16 Hz, H-1), 6.9–7.1 (6 H, aromatic).

Compound **11**. **3** was hydrogenated according to the procedure reported for the preparation of **8**. The residue was purified by CCD between H_2O : AcOEt : n-BuOH 10 : 8 : 2 (K_r = 0.36) and gave sacchariform crystals from EtOH and AcEOt, m.p. 118–120°; MS, *m/e* (%): 625 (1), 316 (78), 184 (27), 152 (54), 138 (100) (Found: C, 58.01; H, 6.54. Calc. for $C_{31}H_{44}O_{14}$: C, 58.11; H, 6.92%).

Compound **12**. Compound **11** was submitted to hydrolysis with cellulase according to the procedure reported for hypoxoside **1**. The aglycone was purified by CCD between H_2O: acetone: cyclohexane 10:10:14 (K_r = 0.68); **11** crystallized as needles from n-hexane and a little quantity of AcOEt, m.p. 77.5–78°; MS, *m/e* (%): 316 (44), 138 (100), 123 (9); ^1H NMR (Varian XL 100), δ: 1.1–1.8 (6 H, H_2-2, H_2-3, H_2-4), 2.52 (4 H, approx. t, H_2-1, H_2-5), 3.83 (6 H, s, 2 OMe), 5.70 (2 H, s exchangeable with D_2O, 2 OH), 6.80 (2 H, dd, J = 2 and 8 Hz, 2 H-6'), 6.82 (2 H, d, J = 2 Hz, H-2'), 7.01 (2 H, d, J = 8 Hz, 2 H-5') (Found: C, 72.17; H, 7.58. Calc. for $C_{19}H_{24}O_4$: C, 72.12; H, 7.65%).

Deuteration of **12**: **13**. A soln of **12** (80 mg) in dimethylformamide (0.5 ml) was added to a soln of NaOD (25 mg) in D_2O (1 ml). After 100 h at 100° in a sealed tube under nitrogen the mixture was treated with H_2O and $CHCl_3$ and the residue of the organic phase was purified by CCD as compound **12**. The solid compound obtained **13**, was washed with n-hexane and dried. MS, *m/e* (%): 318 (46), 139 (100), 123 (10); the aromatic region of ^1H NMR spectrum showed only the split signal at δ 6.81 due to 2 H-2' and 2 H-6'.

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REFERENCES

[1]W. Koenig and E. Knorr, *Chem. Ber.* **34**, 974 (1901).
[2]T. A. Geissmann in The chemistry of flavonoid compounds. Chap. 5, Pergamon Press, Oxford (1962).
[3]J. Klein and S. Brenner, *Tetrahedron* **26**, 5807 (1970).
[4]G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.* 6914 (1965).
[5]K. R. Markham, B. Ternai, R. Stanley, H. Geiger and T. J. Mabry, *Tetrahedron* **34**, 1389 (1978).
[6]D. G. O'Donovan and H. Horan, *J. Chem. Soc.* (*C*) 331 (1971).