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RESEARCH ON AFRICAN MEDICINAL PLANTS - X*

GLUCOSIDES OF HYPOXIS NYASICA BAK. THE STRUCTURE OF NYASOSIDE, A NEW GLUCOSIDE BIOLOGICALLY RELATED TO HYPOXOSIDE

G.B. MARINI-BETTOLO+, M. NICOLETTI and I.MESSANA

Dipartimento di Biologia Vegetale, L.go Cristina di Svezia 24 and Centro dei Recettori e delle Molecole biologicamente attive del C.N.R., Università Cattolica del Sacro Cuore, L.go F. Vito 1, Roma, Italy.

C. GALEFFI

Laboratorio di Chimica del Farmaco, Istituto Superiore di Sanità, V.le Regina Elena 299, Roma, Italy

J.D. MSONTHI

University of Malawi, Zomba, Malawi.

W.A. CHAPYA

International Centre of Insect Physiology and Ecology, Nairobi, Kenya.

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Abstract - From rhizome of Hypoxis nyasica two diglucosides were isolated: hypoxoside, (1), and a new one, the 0,0-β,β-di-D-glucopyranoside of 1-(4'-hydroxyphenyl)-3-(4"-hydroxyphenyl)-1,4-pentadiene, (2), named nyasoside.

In 1981 some of us isolated a glucoside of uncommon structure, named hypoxoside, (1), from the rhizome of Hypoxis obtusa Bush[1] (Hypoxidaceae) collected in Mozambique.

On account of the large utilization of the Hypoxis genus plants in African traditional medicine against urinary infections, prostatic hypertrophy and internal cancer[2,3] we examined Hypoxis nyasica Bak, collected near Zomba (Malawi). From the methanolic extract of rhizome two main glucosides were isolated by counter-current distribution (CCD) between H_2O:AcOEt:n-BuOH, hypoxoside, (1)(5.4%), and a new one, named nyasoside, (2) (3.0%). The latter corresponded to the raw formula $C_{29}H_{36}O_{12}$, m.p. 110-112°C from MeOH and acetone, $(\alpha)_D^{20}$ = -87.0 (c 0.8, MeOH), and showed UV maxima (MeOH) at 208, 257 and 300 (sh) nm (log ε 4.42, 4.07, 3.51) not affected by addition of alkali.

On account of the difficult separation of (2) from (1), (2) could be easily recovered as unaffected by methylation of the mixture with diazomethane whereas hypoxoside was converted into the corresponding dimethyl derivative (3). Like hypoxoside, nyasoside is a diglucoside which gave by acetylation a crystalline octaacetate (4), $C_{29}H_{28}O_{12}(Ac)_8$, m.p. 110-112°C. The mass spectrum of (4) showed the peak due to tetraacetyl hexose (m/z 331, 26%) as well as the corresponding peak at m/z 581 (M^+ -331 m.u., 1%).

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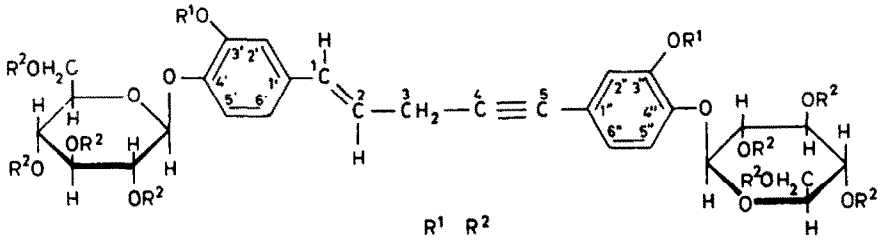
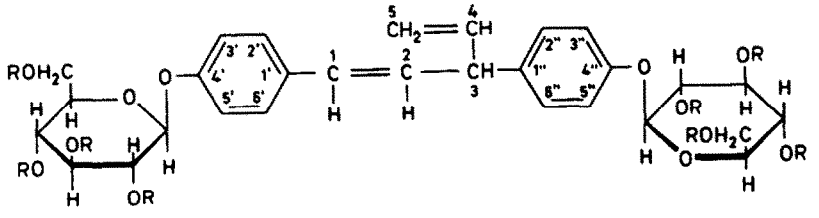
By enzymatic hydrolysis with β-glucosidase, nyasoside gave D-glucose (confirmed through its β-pentaacetate by comparison with an authentic specimen) and an oily aglucone (5), M^+ at m/z 252 (base peak corresponding to $C_{17}H_{16}O_2$, $[\alpha]_D^{20}$ = -147 (c 1, acetone), named nyasol. UV spectrum showed maxima at 206, 258, 297 (sh) nm (log ε 4.41, 4.25 and 3.33) and underwent bathochromic effect with alkali, λ_{max} 274, 297 (sh) nm (log ε 4.26, 3.35). ^{1}H NMR spectrum of (2) (MeOH-d_4, 400 MHz) showed the signals of a vinyl group δ 5.14, dd, J=2 and 16 Hz, and 5.15, dd, J=2 and 10 Hz, AB part, and 6.02, m, X part) and of a vinylene group (δ 5.74, dd, J=11 and 11.2 Hz, and 6.54, d, J=11.2 Hz) whose signals at δ 6.02 and 5.74 were further coupled with a hydrogen at δ 4.51 (broad dd, J=7 and 11 Hz). In the aromatic region of the spectrum a triplet at δ 7.06 (4 H, J=8 Hz) and two doublets at 7.16 and 7.22 (2 H, respectively, J=8 Hz) suggested the presence of two para disubstituted aromatic systems. Two other doublets present at δ 4.92 and 4.95 (J=7.5 Hz) pertained to the anomeric hydrogens of two sugars moieties.

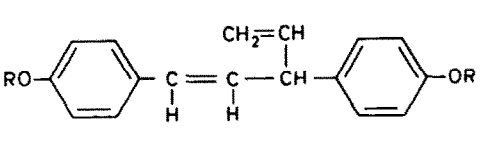
The presence of a 1,4-pentadienic structure in (2) and (5) was confirmed by selective decoupling on ^{1}H NMR spectrum ($CDCl_3$) of the dimethyl ether derivative of the aglucone, (6), $C_{17}H_{14}O_2(Me)_2$, oil, M^+ at m/z 280 (1%). As a matter of fact, by irradiating at δ 6.66 (d, J=11 Hz) the signal at δ 5.80 (t, J=11 Hz) became a sharp doublet (J=11 Hz), whereas by irradiating at δ 4.64 (broad dd) the same signal at δ 5.80 became a doublet (J=11 Hz) and the multiplet at δ 6.10 was simplified into a double doublet (J=10 and 17 Hz) which is further coupled with two hydrogens at δ 5.14 and 5.15. The signals of eight hydrogens between δ 6.8 and 7.4 in (6) were assigned to two O-methylated phenolic systems also in agreement with the mass peaks at m/z 158 (74%, M^+-$C_6H_6O^{\cdot+}$) and at 107 (97%, $C_7H_7O^+$) for (5) and the corresponding at m/z 172 (22%) and 121 (44%) for (6).



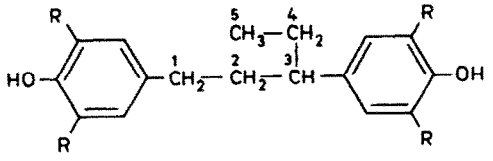
	R^1	R^2
1:	H	H
3:	Me	H
9:	Ac	Ac



2: R=H
4: R=Ac



5: R=H
6: R=Me



7: R=H
8: R=D

The coupling constant $J_{1',2}$ in (2) and (6) (11.2 and 11 Hz, respectively) is at the upper limit of the cis value as electronegative substituents, such as phenyl groups, raise the typical value[4]. The para position of the two hydroxy groups of the aromatic rings was confirmed according to the procedure described by Kirby and Ogunkoya[5] carried out on the tetrahydro derivate of (5), (7), $C_{17}H_{20}O_2$, m.p. 102-103°C, M^+ at m/z 256 (18%), $(\alpha)_D^{20}$=-41.5 (c 1, MeOH). In fact, by exchange with alkaline deuterium oxide, four hydrogen atoms, in ortho position to the hydroxy groups, were replaced by four deuterium atoms in the derivative obtained (8). Accordingly the two AA'BB' systems of (7) were made into two signals at δ 6.93 (2 H) and 6.98 (2 H) in (8), and in the ^{13}C NMR spectrum of the latter the signals of C(3'), C(3"), C(5') and C(5") (overimposed at 115.0 ppm) displayed a dramatic lowering in comparison with the spectrum of (7) (see table 1).

Table 1. ^{13}C NMR chemical shift assignments[a].

Compound	(2)[b]	(4)[c]	(5)	(6)	(7)
Solvent	CD_3OD	$CDCl_3$	CD_3COCD_3	$CDCl_3$	$CDCl_3$
C(1)	132.5	132.1	131.6	131.4	32.5
C(2)	130.7	128.4	129.0	128.3	38.4
C(3)	48.1	46.8	47.5	46.5	46.3
C(4)	142.1	140.1	141.9	140.5	29.9
C(5)	115.2	115.3	114.3	113.4	12.0
C(1')	133.1	132.7	133.3	131.4	134.6
C(1")	138.5	138.0	134.6	140.5	137.5
C(2'), C(6')	130.7	129.7	130.3	129.5	129.2
C(2"), C(6")	129.5	128.6	129.0	128.5	128.6
C(3'), C(5')	118.0[d]	117.0[d]	115.6[d]	114.7[d]	115.0
C(3"), C(5")	117.4[d]	116.6[d]	115.2[d]	115.1[d]	115.0
C(4')	157.5[e]	155.5[e]	156.3[e]	154.2	153.0[d]
C(4")	157.9[e]	155.7[e]	156.9[e]	154.2	153.2[d]
OMe				55.0	

[a] Chemical shifts in ppm downfield from TMS.

[b] Signals of glucose: 102.5 and 102.0, C(1); 74.9, C(2); 77.9, C(3) or C(5); 71.4, C(4); 77.8, C(5) or C(3); 62.5, C(6).

[c] Signals of glucose: 99.0 and 98.8, C(1); 71.0, C(2); 72.6, C(3) or C(5); 68.2, C(4); 71.9, C(5) or C(3); 61.8, C(6). Acetyl groups: 170.2, 170.0, 169.1, 169.0 (COMe); 20.5 (COMe).

[d,e] Signals may be interchanged in the same column.

Therefore the structure (5) was unambiguously assigned to nyasol, aglucone of (2). For the nyasoside the β-glucosidic linkage was established by the coupling constant value of the anomeric hydrogens and by easy hydrolysis with β-glucosidase, whereas the β-pyranosidic structure was established by comparison of ^{13}C NMR signals of (4) with the corresponding ones of phenyl-β-glucopyranosides[6].

The ^{13}C NMR data of (2) and (4)-(7) (table 1) were in good agreement with the assigned structures; in particular it was possible to confirm the presence of the vinyl group in (4) - (6) (ca. 114 ppm for C(5) and ca. 140 for C(4)) and the para substition of the two aromatic rings owing to the identity of the signals of C(2'), C(3'),

C(2"), C(3") with C(6'), C(5'), C(6"), C(5"),respectively. The distinction between the signals of C(1') (132.1 ppm) and C(1") (138.0 ppm) in (4) was made by comparison with that of C(1') (132.7 ppm) in decaacetylhypoxoside, (9)[1].

In agreement with the presence of C(3) chiral center of (2), compounds (4) and (6) displayed a Cotton effect at ca. 240 nm, whose negative sign it was impossible to correlate to the absolute configuration on account of the absence of proper models.

Hypoxoside, (1), and nysoside, (2), are natural products of uncommon structure. Their aglucones can be considered derivative by junction of a C_6-C_2 unit (acetylenic in (1), and olefinic in (2)) on a C_6-C_3 moiety, in γ position in the propenylic system of (1), and in α position in the allylic system of (2).

EXPERIMENTAL

A Craig Post apparatus (200 stages, 10: 10 upper and lower phase) was used for CCD. The separations were monitored by tlc analysis on silica gel F_{254}. Solvent 1: n-BuOH: AcOH:H_2O 4:1:5 (upper phase); solvent 2: AcOEt:toluene 1:1; solvent 3: $CHCl_3$:AcOEt 6:4. Spots were detected by short wave UV light and by anisaldehyde-sulphuric acid spray reagent, if not otherwise reported. Mass spectra were obtained on an LKB 2091 spectrometer. ^1H and ^{13}C NMR spectra were recorded with a Varian XL 100 spectrometer if not otherwise reported (TMS as internal reference). β-glucosidease is β-glucosidase EG. 3.2.121 (Fluka). ORD curves were registered with a Cary 60 spectrophotometer.

Material and extraction. A rhizome of Hypoxis nyasica was collected near Zomba (Malawi). The dried material (67 g) was ground and exhaustively extracted with MeOH. The residue of the extract (20 g) was dissolved in water (150 ml) and the solution was extracted with n-BuOH sat. with H_2O (3x150 ml). The residue of the pooled butanolic extract amounted to 12 g.

Separation. Part of the residue (4 g) was submitted to CCD (200 transfers) between H_2O:AcOEt:n-BuOH 5:4:1, (tlc, solvent 1). The substances were recovered from aqueous phase by extraction with n-BuOH. Two main fractions at K_r = 1.0 (2 g) and K_r = 0.33 (0.11 g) were obtained and only the former was here examined. It was again submitted to CCD operating on recycling (1000 transfers) between H_2O:AcOEt:n-BuOH (4:3:1). Two substances, having the same Rf value, (1) (K_r = 1.78, 1.2 g, 5.4% of the rhizome) and (2) (K_r = 1.39, 0.67 g, 3.0%) were obtained and only the former gave positive reaction for phenols (potassium ferricyanide and ferric chloride).

Hypoxoside. Substance (1), crystallized from abs. EtOH by rubbing with glass rod, was identified as hypoxoside by direct comparison as well as by comparison of the corresponding dimethyl (3) and decaacetyl (9) derivatives (m.p., rotatory power and spectroscopic data[1].

Nyasoside. Substance (2) crystallized slowly from MeOH and acetone, m.p. 110-112°C, UV (MeOH) λ_{max}: 208, 257, 300 (sh) nm (log ε 4.42, 4.07, 3.51); IR (KBr): 3360 (broad) and 1600 cm^{-1}; $(\alpha)_D^{20}$ = -87.0 (c 0.8, MeOH). (Found: C, 59.67; H, 6.06. Calc. for $C_{29}H_{36}O_{12}$: C, 60.41; H, 6.29%). ^1H NMR (MeOH-d_6, Bruker AM-400): 3.30-3.95 (12 H, H-2' - H-6 of the sugar moieties), 4.51 (1 H, bdd, J=7 and 11 Hz, H-3), 4.92 and 4.95 (1 H respectively, d, J=7.5 Hz, anomeric hydrogens of the sugar moieties), 5.14 (1 H, dd, J=2 and 16 Hz, H_A-5), 5.15 (1 H, dd, J=2 and 10 Hz, H_B-5), 5.74 (1 H, dd, J=11.0 and 11.2 Hz, H-2), 6.02 (1 H, m, H-4), 6.54 (1 H, d, J=11.2 Hz, H-1), 7.04-7.22 (8 H, aromatic protons).

In order to avoid the tedious separation of (2) from (1), the mixture was submitted to methylation with ethereal CH_2N_2 in MeOH for 4 days. By subsequent CCD purification (H_2O:AcOEt:n-BuOH 4:3:1, tlc, solvent 1) dimethylhypoxoside, (3), (K_r = 0.43) m.p. 157-159° C (abs. EtOH)[1] and unaffected nyasoside, (2), (K_r = 1.4) were easily separated.

Octaacetyylnyasoside (4). A mixture of hypoxoside, (1), and nyasoside, (2), was acetylated with a mixture of pyridine and Ac_2O (1:1). After a day, the reagents were evaporated under vacuum and the two acetyl derivatives (tlc, solvent 2) were separated by CCD (H_2O:acetone:EtOH:AcOEt:cyclohexane 7:4:3:1:10, 200 transfers) and reco-

vered from lower phase by extraction with $CHCl_3$. Decaacetylhypoxoside, (9), K_r = 0.33) crystallized from AcOEt and n-hexane, m.p. 127-128° C Octaacetylnyasoside, (4), (K_r = 1.63) m.p. 110-112° C from EtOAc, $(\alpha)_D^{20}$ = -82.5 (c 1.5, $CHCl_3$) showed UV maxima at λ 204, 254, 284 (sh) nm (log ε 4.58, 4.29, 3.52). ^{1}H NMR ($CDCl_3$), δ : 2.08 (24 H, s, 8 Ac), 4.22 (4 H, m; 2 $\underline{CH_2}$OAc), 4.55 (1 H, broad dd, J=7 and 11 Hz, H-3), 4.9-5.4 (12 H, 10 monose methynes and H_2-5), 5.80 (1 H, t, J=11 Hz, H-2), 6.01 (1 H, m, H-4) 6.60 (1 H, d, J=11 Hz, H-1), 6.9-7.4 (8 H, aromatic). MS, m/z (%): 581 (1), 331 (26), 43 (100); ORD (MeOH), [Φ] (λ_{max}, nm): +82600 (220), -12000 (260); (Found: C, 59.38; H, 5.59. Calc. for $C_{45}H_{52}O_{20}$: C, 59.20, H, 5.74%).

Hydrolysis of nyasoside with β-glucosidase

Aglycone: nyasol (5). Acetate buffer soln at pH 5.5 (40 ml) was added to an aqueous soln (40 ml) of (2) (300 mg) and of β-glucosidase (15 mg). The soln, covered by toluene and allowed to stand at 36° C, separated an oil after 2 h. After a night the hydrolysis was completed (tlc, solvent 1). Some drops of AcOH were added, then AcOEt was added to dissolve the oil and extract the aqueous phase. The residue of the organic phase was purified by CCD (100 stages), solvents: H_2O:acetone:cyclohexane:AcOEt 5:5:5:1 (tlc, solvent 3, detection with potassium ferricyanide-ferric chloride) and recovered from the lower phase by extraction with AcOEt. The oily aglucone (5) (K_r = 2.5, 104 mg) resisted any attempt at crystallization. $(\alpha)_D^{20}$ = -147 (c 1, acetone); UV (MeOH) λ_{max} 206, 258, 297 (sh) nm (log ε 4.41, 4.25, 3.33) (OH^-) 274, 297 (sh) nm (4.26, 3.35); MS, m/z (%): 252 (M^+, $C_{17}H_{16}O_2$, 100), 238 (37, M^+-CH_2), 158 (74), 107 (97); ^{1}H NMR ($CDCl_3$), δ : 4.42 (1H , bdd, J=7 and 12 Hz, H-3), 5.14 (1 H, dd, J=2 and 10 Hz, H_A-5), 5.15 (1 H, dd, J=2 and 16 Hz, H_B-5), 5.65 (1 H, t, J=11 Hz, H-2), 5.96 (1 H, m, H-4), 6.50 (1 H, d, J=12 Hz, H-1), 6.7-7.1 (8 H, aromatic).

Identification of the sugar. After AcOEt extraction, the aqueous phase of the hydrolysis 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 by comparison of IR and ^{1}H NMR spectra and rotatory power with an authentic specimen of β-D-pentaacetylglucose[7].

Dimethylnyasol (6). Nyasol (5) dissolved in MeOH was methylated with an ethereal soln of CH_2N_2. After 1 day, the solvents were evaporated and the residue was purified by CCD between H_2O:EtOH:acetone:n-hexane 2:2:1:4 (K_r = 0.66, tlc, solvent 2). The oily compound resisted any attempt at crystallization. $(\alpha)_D^{20}$ = -203.1 (c 0.5, acetone); MS; m/z (%): 280 (M^+, $C_{19}H_{20}O_2$, 1), 266 (100), 251 (33), 172 (22), 158 (57), 121 (44), 107 (43); ^{1}H NMR ($CDCl_3$), δ : 3.92 (6 H, s, 2 OMe), 4.64 (1 H, bdd, J=7 and 11 Hz, H-3), 5.14 (1 H, dd, J=2 and 10 Hz, H_A-5), 5.15 (1 H, bdd, J=2 and 17 Hz, H_B-5), 5.80 (1 H, t; J=11 Hz, H-2), 6.10 (1 H, m, H-4), 6.66 (1 H, d, J=11 Hz, H-1), 6.8-7.4 (8 H, aromatic); ORD (MeOH), [Φ] (λ_{max}, nm): +26000 (220), -22500 (260).

Tetrahydronyasol (7). By hydrogenation in presence of $Pt/BaSO_4$ 5% (70 mg) compound (5) (100 mg), dissolved in 90% aqueous MeOH (10 ml), absorbed within 1 h 2 hydrogen moles/mole. After 1 night, the catalyst was removed by filtration and the dried residue of the soln was purified by CCD between H_2O:acetone:cyclohexane 4:6:7 (K_r = 1.2, tlc, solvent 3). The substance was recovered from the lower phase by extraction with EtOAc. (7) crystallized from n-hexane, m.p. 102-103° C; $(\alpha)_D^{20}$ = -41.5 (c 1, MeOH); MS, m/z (%) 256 (M^+, 18), 227 (3), 135 (30), 107 (100); ^{1}H NMR ($CDCl_3$), δ : 0.72 (3 H, t, J=7 Hz, H_3-5), 1.2-1.9 (4 H, H_2-2 and H_2-4), 2.1-2.6 (3 H, H_2-1 and H-3), 6.04 (2 H, exchangeable with D_2O, 2 OH), 6.7-7.1 (8 H, aromatic). (Found: C, 79.84; H, 7.72. Calc. for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86%).

Deuteration of (7): (8). A soln of (7) (41 mg) in dimethylformamide (0.4 ml) was added to a soln of NaOD (32.8 mg) in D_2O (1 ml). After 100 h at 100° C 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 tetrahydronyasol. The solid compound obtained, (8), was washed with n-hexane and dried. MS, m/z (%): 260 (14), 231 (2), 137 (44), 109 (100). The aromatic region of ^{1}H NMR spectrum of (8) showed only the two singlets at δ 6.93 and 6.98 assignable to 2', 6' and 2", 6".

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