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RESEARCH ON AFRICAN MEDICINAL PLANTS - XIII[+]

NYASICOSIDE, A NEW GLUCOSIDE OF HYPOXIS NYASICA BAK.

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ABSTRACT - From the rhizome of Hypoxis nyasica a new monoglucoside, the 1-(3',4'-dihydroxyphenyl)-5-(3'',4''-dihydroxyphenyl)-1-hydroxy-2-O-β-D-glucopyranosyl-pentan-4-yne named nyasicoside, was isolated and its structure elucidated by chemical and spectroscopic methods.

Hypoxis nyasica Bak is a Hypoxidacea of Southern Africa and its rhizomes are used in traditional medicine against urinary infections and prostatic hypertrophy [1].

In a previous work on a sample of plant collected near Zomba (Malawi) we reported the identification of two new glucosides of uncommon structures: hypoxoside, 1, previously isolated from H.obtusa Busch [2], and nyasoside, 2 [3, 4]. On account of the very similar chromatographic behaviour of the two compounds, the latter had been obtained as unaffected after methylation of the mixture with diazomethane and separation by counter-current distribution (CCD), together with dimethylhypoxoside, 3, and the methyl derivative of a minor compound.

This methyl derivative, 4, now available in larger amount, m.p. 196-7° C, corresponds to the raw formula $C_{27}H_{34}O_{11}$ (M+ m/z 534, 26%), $[\alpha]_D^{20}$ = +19.6 (MeOH), UV λmax, nm (log ε): 297 (3.72), 285 (3.87), 258 (4.38). Its ^{1}H NMR spectrum ($CDCl_3$+CD_3OD) showed the presence of four aromatic methoxy groups (δ 3.85 (1), 3.86 (x2), 3.89 (1)) and of two equally substituted aromatic systems, each of them consisting of two ortho protons, one of which showed an additional meta coupling with another aromatic proton, in agreement with the IR band at 819 cm^{-1}, corresponding to the 1,2,4 trisubstituted aromatic system. These data suggested the hypothesis of the presence in the non-methylated compound of four free hydroxy groups symmetrically arranged, as two pairs in an ortho relationship, and therefore the possibility of its separation from 1 and 2 by precipitation with lead acetate in aqueous solution and the subsequent recovery of the free compound by exchange with hydrogen sulfide. As a matter of fact, this procedure allowed the isolation of this new compound, named nyasicoside, 5, m.p. 120-2°C, which corresponds to the raw formula $C_{23}H_{26}O_{11}$, M+ m/z 478. The four hydroxy groups are indeed symmetrically arranged, as two ortho pairs, as suggested by the bathochromic shift in the UV spectrum on addition of AcONa and H_3BO_3 and confirmed by ^{1}H NMR data (see Table 1). Moreover, the same ^{1}NMR spectrum showed the additional signals of two vicinal protons at δ4.54, d, J = 7.0 Hz, and 3.78, m, the last of which has an additional coupling with two geminal protons, respectively, at δ 2.20 (dd, J = 6.0 and 17.0 Hz) and 2.43 (dd, J = 4.0 and 17.0 Hz).

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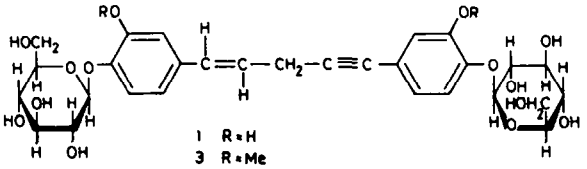

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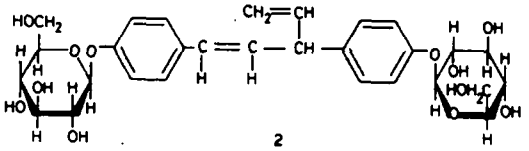
3 R=Me



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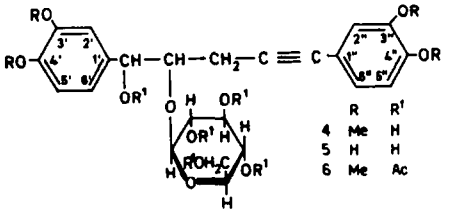
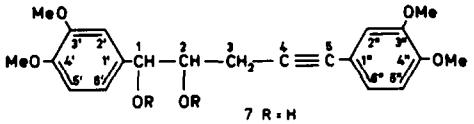


	R	R'
4	Me	H
5	H	H
6	Me	Ac



7 R=H
8 R=Ac

The acetylation and the hydrolysis of the aforementioned tetramethyl derivative of 5, 4, $C_{23}H_{22}O_7(OMe)_4$, accounted for the remaining hydrogens. Thus by acetylation of 4 with pyridine and Ac_2O, the pentaacetyl derivative, 6, $C_{23}H_{17}O_2(OMe)_4(OAc)_5$, m.p. 69-71°C, $[\alpha]_D^{20}$ = +6.5, was obtained. Among the signals of the above-mentioned sequence CH-CH-CH_2, only the doublet at lower field underwent remarkable deshielding (δ 4.64 to 5.94) due to the acetylation of an adjacent hydroxy group. The vicinal proton presumably belongs to a carbon bearing an O-glucosidic linkage with a hexose whose five protons (two belonging to the CH_2OH) bore the deshieding effect of the acetylation (see Table 1). The two remaining carbon atoms of 5 belong to an acetylenic bond completely substituted as it ensued from: i) the presence in the ^{13}C NMR spectra of 4-6 (see Table 2) of two signals between 80 and 85 ppm, as in hypoxoside, 1, [2] singlets in the SFORD spectra, ii) the presence in 4 of a weak IR band at 2230 cm^{-1}, iii) the absence of other ^1H NMR signals in δ 2.3-3.0 region.

The sequence of the five carbon atoms in the central chain, and in particular the position of the methylene group adjacent to the triple bond, as in hypoxoside, 1, was assigned on the basis of the chemical shift for the two geminal protons which turned out the alternative benzylic position. In agreement with this structure, the following peaks originated from the molecular ion are present in the mass spectrum of 4: the couple at m/z 371 (28%) and 372 (18) due to the loss of the phenylacetylenic moiety, the peak at m/z 354 (50) due to the loss of the whole sugar unit and the set at m/z 206 (46), 205 (42) and 204 (76) due to the benzylic cleavage and the loss of the monose unit. In agreement with the O-glucosidic structure of nyasicoside, its tetramethyl derivative, 4, was hydrolyzed by β-glucosidase giving rise to D-glucose (identified by tlc and through the corresponding β-pentaacetate) and the optically active aglucone, tetramethylnyasicol, 7, oil, $[\alpha]_D^{20}$ = +85.5(MeOH), M+ m/z 372 (18%), corresponding to the raw formula $C_{17}H_{12}O_2(OMe)_4$. The two vicinal hydrogens of the diole moiety (δ 3.80, m, and 4.50, d) underwent deshielding effect by subsequent acetylation (δ 5.34, m, and 6.00, d, respectively) in the corresponding diacetyl derivative, 8, $C_{17}H_{10}(OMe)_4(OAc)_2$, oil, M+ m/z 456 (71%).

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

Compound	5	4 [b]	6 [c]	7 [d]	8 [e]
H					
1	4.54, d J = 7.0	4.64	5.94	4.50	6.00
2	3.78, m	4.07	ov.	3.80	5.34
3	2.20, dd J = 17.0 and 6.0	2.41	2.19	2.25	2.41
	2.43, dd J = 17.0 and 4.0	2.63	2.50	2.42	2.62
2',2''	6.66, d J = 2.0	6.95 and 7.01	6.80 and 6.89	6.79 and 6.99	6.85 and 6.88
5',5''	6.57, d J = 8.0	6.83 and 6.90	7.12 and 7.30	6.68 and 6.72	6.74 and 6.79
6',6''	6.74, dd J = 8.0 and 2.0	6.80 and 7.08	6.90 and 6.93	6.83 and 6.88	6.95 and 6.97
glucose					
1	4.53, d J = 7.0	4.78	4.82		
2	ov.	3.1 - 3.5	5.01, dd J = 9.0 and 7.0		
3	3.31, t J = 9.0	3.1 - 3.5	5.07		
4	3.48, t J = 9.0	3.1 - 3.5	5.18		
5	ov.	3.1 - 3.5	ov.		
6	3.60, dd J = 12.0 and 2.0	3.73	4.08		
	3.78, dd J = 12.0 and 4.5	3.82	4.26		

[a] Coupling constant values are in Hz and chemical shifts in δ. In $CD_3OD:CDCl_3$ 2:8, except 6 and 8 in $CDCl_3$. ov. = overlapped with other signals.
[b] OMe: 3.85, 3.86 (x2) and 3.89. [c] OMe: 3.82, 3.84 (x2) and 3.85; OCOMe: 1.90, 1.96, 2.00, 2.08 and 2.09. [d] OMe: 3.86 (x4). [e] OMe: 3.80 (x4); OCOMe: 2.06 (x2).

Table 2. ^{13}C NMR chemical shift assignments. [a]

Compound	4	5	6	7	8
1	77.2	75.9	75.3	75.8	75.4
2	83.1	82.8	83.5	73.9	72.7
3	22.9	22.7	21.9	23.8	21.9
4	84.8	83.8*	83.8	84.2	81.3
5	82.7	83.3*	79.8	82.0	82.3
1'	116.3	115.1	115.6	116.0	115.8
1''	133.2	132.2	128.8	133.6	131.9
2',2''	111.2°, 111.8°	114.8°, 115.7°	111.0°, 111.2°	110.1°, 111.1°	110.3°, 110.9°
3',3'', 4',4''	149.5, 149.2	145.2, 145.3 145.9	149.7, 149.8 150.1	148.3, 148.5 148.8	149.2, 149.3
5',5''	111.8°, 115.2°	115.7°, 119.0°	111.2°, 114.5°	111.2°, 114.5°	111.0°, 114.3°
6'	120.5	119.7	119.7	119.1	119.9
6''	125.3	124.5	124.7	124.5	124.6
OMe	56.2		55.7	55.4	55.7
OCOMe			169.3,169.4,169.7 170.2, 170.6		169.6
OCOMe			20.6, 21.1		20.9
glucose					
1	102.8	102.8	100.5		
2	74.1	74.1	71.2*		
3	76.9*	77.0$^{\Delta}$	72.7*		
4	70.6	70.6	69.3		
5	76.1*	76.8$^{\Delta}$	71.6*		
6	62.0	61.9	61.9		

[a] Chemical shift values in ppm downfield from TMS. In $CD_3OD:CDCl_3$ 2:8, except 6 and 8 in $CDCl_3$.
°,*,$^{\Delta}$ These signals may be interchanged in the same column.

Like hypoxoside, nyasicoside shows a C_6-C_3-C_2-C_6 system, but in the latter the olefinic function of the 1-en-4-yne structure of the former is hydroxylated to diol. Moreover, differently from hypoxoside and nyasoside, the sugar unit in 5 is linked to an alcoholic instead of a phenolic function. The configurations of the two chiral centers, C(1) and C(2), are not determined, owing to the scarcity of the product hitherto obtained; however, nyasicoside and its derivatives show a broad positive Cotton effect in CD with maxima at 245 nm ($[\Theta]$ = +8000 in 5 and +25000 in 4) not correlatable to the absolute configuration.

EXPERIMENTAL

A Craig Post apparatus (200 stages, 10:10 ml, upper and lower phase) was used for CCD. The separation, as well as the rate of reactions, 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. Mass spectra of 7 and 8 were registered with an LKB 2091 spectrometer, whereas mass spectra of 4 and 5 were registered with an EI Kratos MS 30 spectrometer at the Department of MS of the Consiglio Nazionale delle Ricerche (University of Naples), to which the authors are greatly indebted. ^1H and ^{13}C NMR spectra were recorded with a Bruker AM 400 spectrometer (TMS as internal reference). The circular dicroism (CD) curves were registered with a Jasco J-40 apparatus. ^1H and ^{13}C NMR spectral data of compounds 4-8 are reported in tables 1 and 2, respectively.

Plant material, extraction and separation-Rhizomes of H.nyasica Bak were collected near Zomba (Malawi) in February 1986. The dried material (130 g) was ground and exhaustively extracted with aqueous MeOH (80%) and the eluate was then evaporated. The extract (45 g) was submitted by 9 g portions to CCD between H_2O: AcOEt: n-BuOH 10:7.5:2.5 and 15 g of mixture A at Kr 1 were obtained. 7.5 g of (A) were submitted to methylation with ethereal diazomethane in MeOH for 4 days. By subsequent CCD separation (H_2O: EtOH: AcOEt: cyclohexane 9:4:13:1; tlc, solvent 1) tetramethylnyasicoside, 4, (Kr = 0.52, 0.38 g), nyasoside, 2, and dimethylypoxoside, 3, were obtained. The remaining fraction of (A) was dissolved in water (100 ml) and mixed with a 5% aqueous solution of lead acetate (25 ml). The collected precipitate of lead salts was washed with water (2x15 ml) and then, suspended in water (100 ml), was submitted to a stream of hydrogen sulfide for the recovery of the free ortho diphenolic compound. After the separation of lead sulfide and concentration of the solution, nyasicoside, 5, was finally purified by CCD (H_2O: AcOEt: n-BuOH 10:8:2, Kr = 0.83, 0.32 g).

Nyasicoside, 5 - The compound crystallized from EtOH and AcOEt, m.p. 120-2°C, UV (MeOH) λmax: 257, 290, 302 nm (log ε 4.22, 3.82, 3.66), +AcONa and H_3BO_3: 264, 296, 307 (sh) (log ε 4.15, 4.00, 3.80); IR (KBr): 3400 (broad), 1600, 812 cm^{-1}; $[\alpha]_D^{20}$ = +14.7 (c 0.9, MeOH); MS, 478 m/z (M+); CD (MeOH), $[\Theta]$ (λmax, nm): +8000 (245). (Found: C, 57.35; H, 6.04. Calc. for $C_{23}H_{26}O_{11}$: C, 57.74; H, 5.48%).

Tetramethylnyasicoside, 4 - Crystals from EtOH and AcOEt, m.p. 196-7°C, UV (MeOH) λmax: 258, 285, 297 nm (log ε 4.38, 3.87, 3.72). IR (KBr): 3400 (broad), 2230, 1600, 819 cm^{-1}; $[\alpha]_D^{20}$ = +19.6 (c 1.2, MeOH); MS, m/z (%): 534 (M+, 26), 372 (18), 371 (28), 354 (50), 206 (46), 205 (42), 204 (76), 175 (100); CD (MeOH), $[\Theta]$ (λmax, nm): +25000 (245). (Found: C, 60.37; H, 6.73. Calc. for $C_{27}H_{34}O_{11}$: C, 60.66; H, 6.41%).

Tetramethyl-pentaacetylnyasicoside, 6 - Tetramethylnyasicoside, 4, was acetylated with a mixture of pyridine and Ac_2O (1:1). After one day the reagents were evaporated under vacuum and the residue was crystallized from n-hexane, m.p. 69-71°C, $[\alpha]_D^{20}$ = +6.5 (c 0.8, MeOH) (Found: C, 59.42; H, 5.78. Calc. for $C_{37}H_{44}O_{16}$: C, 59.67; H, 5.96%).

Hydrolysis of tetramethylnyasicoside, 4.

Tetramethylnyasicol, 7 - Acetate buffer soln at pH 5.5 (15 ml) was added to an aqueous soln (40 ml) of 4 (80 mg) and β-glucosidase (EG.3.2.121 (Fluka)) (15 mg). The soln was covered by toluene and allowed to stand at 36°C. After two days, the hydrolysis was incomplete (tlc, solvent 1) and more enzyme (10 mg) was added. After four days the tlc analysis showed the fulfilment of the reaction. The soln was then extracted with AcOEt (50 mlx2) and the residue of the organic phase was submitted to CCD (H_2O:acetone:cyclohexane:AcOEt 5:5:5:2). The oily aglucone, 7, cromatographycally unitary (solvent 2), (Kr 0.38, 30 mg) resisted any attempt at crystallization. $[\alpha]_D^{20}$ = +85.5 (c 1.1, MeOH). MS, m/z: 372 (M+, $C_{21}H_{24}O_6$, 18), 206 (18), 166 (100).

Identification of the sugar - After the AcOEt extraction, the aqueous phase of the hydrolysis was extracted with n-BuOH and then percolated through a column of Dower 50 W (H+). In the residue D-glucose was identified by tlc (solvent: H_2O: MeOH: AcOH: ethylene dichloride 2:3:5:10) and through the corresponding β-pentaacetate by comparison with an authentic specimen of β-D-pentaacetylglucose.

Acetylation of tetramethylnyasicol, 8 - Compound 7 was acetylated according to the routine procedure. Oil. MS, m/z (%): 456 (M+, $C_{25}H_{28}O_8$, 71), 397 (23), 355 (62), 354 (41), 337 (82), 336 (68), 166 (100).

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