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RESEARCH ON AFRICAN MEDICINAL PLANTS. XXVII*. INTERJECTIN, A DERIVATIVE OF NYASICOSIDE FROM HYPOXIS INTERJECTA AND HYPOXIS MULTICEPS

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Abstract - Interjectin, isolated from the rhizomes of H. interjecta and H. multiceps, is a diglucoside of a norlignan, nyasicol, plus an additional p-hydroxy cinnamoyl unit. The structure was correlated to that of tetramethylnyasicoside.

In 1982 the structure of the first norlignan, hypoxoside, 1, isolated from the rhizomes of a Hypoxidacea, Hypoxis obtusa Burch [1], was reported. Later other Ph-C_5-Ph glycosides were isolated from plants of the same family, which are reported for the treatment of prostatic hypertrophy and cancer in African traditional medicine. Thus nyasoside [2], mononyasines A and B [3], nyasicoside, 2 [4], and nyaside [5] were isolated from H. nyasica, obtuside A, 3, and obtuside B, 4 [6], from H. obtusa. In this paper the rhizomes of two South-African Hypoxis, H. interjecta Nel and H. multiceps Buching ex Krauss, which showed identical HPLC profiles, are examined. By counter-current distribution (CCD) of the methanolic extract, the two monoglucosides, obtusides A, 3, and B, 4, hypoxoside, 1, a mixture of triglucosides of their aglucone, 5, and a new glucoside, named interjectin, were isolated.
Interjectin, 6, corresponds to raw formula $C_{38}H_{42}O_{18}$, FAB mass spectrum (negative ion mode): m/z 785 $[M-H]^-$, 623 $[M-163]^-$. It was made into a trimethyl derivative, 7, (δ 3.84, 3.87 and 3.88, 3 OMe), $C_{32}H_{42}O_{16}$, by methylation with diazomethane. The loss of $C_9H_6O_2$ respect to 6 is due to

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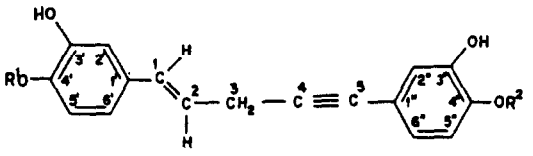
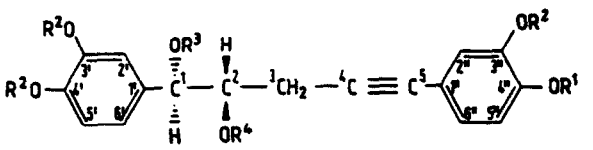
the loss of p-hydroxy cinnamic acid in the methylation. In fact the ^{1}H NMR spectrum of 7 (Table 1) is lacking in a AA'BB' aromatic system (δ 6.7-6.8 and 7.12, 4H), and a trans vinylene system (6.20, H_{α}, and 7.57, H_{β}, J=16.0 Hz). Also the ^{13}C NMR spectrum of 7 (Table 2) and the hypsochromic effect after the methylation (see UV spectral data of 6 and 7, Experimental) gave account of this loss respect to 6. The ion at m/z 623 in the FAB mass spectrum of 6 is due to the loss of the same p-hydroxy cinnamoyl moiety.

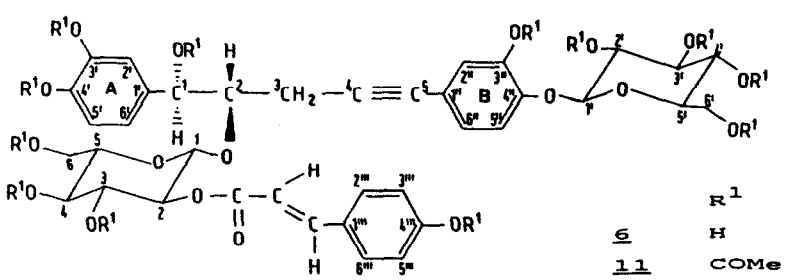
The same trimethyl derivative, 7, by hydrolysis with β-glucosidase gave glucose, which was identified through the corresponding β-pentaacetate, and an aglucone, 8, corresponding to raw formula $C_{20}H_{22}O_6$, M^+ at m/z 358.



	R^1	R^2
1	Gl	Gl
3	Gl	H
4	H	Gl
5	H	H



	R^1	R^2	R^3	R^4
2	H	H	H	Gl
7	Gl	Me	H	Gl
8	H	Me	H	H
9	H	H	H	H
10	Me	Me	H	H
12	H	H	H	p-hydroxy cinnamoyl→2-Gl
13	Me	Me	H	Gl



	R^1
6	H
11	COMe

Table 1. ^{1}H NMR chemical shift assignments of 6-8 and 11-12 #.

	6	7	11*	12	8
H-1	4.70,d J=7.0	4.87,d[a] J=7.5	6.10,d J=6.0	ov.	4.66,d J=7.0
H-2	4.22,m	3.3-3.8	4.04,m	3.94,m	4.0, ov.
H-3	2.42,dd J=17.0,3.0 2.18,dd J=17.0,7.0	2.60,dd J=17.0,4.5 2.40,dd J=17.0,5.5	2.49,dd J=17.0,4.5 ov.	2.38,dd J=17.0,3.0 2.20,dd J=17.0,7.0	2.59,dd J=17.0,4.0 2.44,dd J=17.0,6.0
H-2'	6.85,d[a] J=2.0	7.07,d[b] J=2.0	7.18,d[a] J=2.0	6.86,d J=2.0	6.89,d[a] J=2.0
H-5'	6.7-6.8[b]	6.89,d[c] J=8.0	7.11,d[b] J=8.0	7.16,d[a] J=8.0	6.81,d[b] J=8.0
H-6'	6.7-6.8	6.96,dd[d] J=8.0,2.0	7.24,dd[c] J=8.0,2.0	6.71,dd[b] J=8.0,2.0	6.9-7.0
H-2"	6.90,d[a] J=2.0	6.99,d[b] J=2.0	7.03,d[a] J=2.0	6.86,d J=2.0	6.9-7.0[a]
H-5"	6.93,d[b] J=8.0	7.06,d[c] J=8.0	6.60,d[b] J=8.0	6.65,d[a] J=8.0	6.83,d[b] J=8.0
H-6"	6.7-6.8	7.00,dd[d] J=8.0,2.0	6.91,dd[c] J=8.0,2.0	6.7-6.8[b]	6.9-7.0
H-2"',6"'	7.12,d J=8.0		7.17,d J=8.0	6.78,d J=8.0	
H-3"',5"'	6.7-6.8		6.96,d J=8.0	6.72,d J=8.0	
H-α	6.20,d J=16.0		6.01,d J=16.0	6.15,d J=16.0	
H-β	7.57,d J=16.0		7.41,d J=16.0	7.60,d J=16.0	
OMe		3.88,3.87 3.84			3.86,3.84 3.81
glucose					
H-1	4.87,d J=8.0	4.74,d[a] J=7.5	4.86,d J=8.0	4.93,bs	
H-2	4.95,dd J=9.0,8.0	3.3-3.8	5.1-5.4	4.94,bd J=8.0	
H-3	3.3-4.0	3.3-3.8	5.1-5.4	3.69,bt J=9.0	
H-4	3.3-4.0	3.3-3.8	5.1-5.4	3.52,t J=9.0	
H-5	3.3-4.0	3.3-3.8	3.77,m[d]	3.42,m	
H-6	3.3-4.0	3.3-3.8	4.2-4.3,m	3.95,dd J=12.0,2.0	
			4.13,dd[e] J=12.0,2.0	3.77,dd J=12.0,5.0	
H-1'	4.52,d J=8.0	ov.	5.11,d J=8.0		
H-2'	3.3-4.0	3.3-3.8	5.1-5.4		
H-3'	3.3-4.0	3.3-3.8	5.1-5.4		
H-4'	3.3-4.0	3.3-3.8	5.1-5.4		
H-5'	3.3-4.0	3.3-3.8	3.93,m[d]		
H-6'	3.3-4.0	3.3-3.8	4.2-4.3,m		
			4.08,dd[e] J=12.0,2.0		

#Coupling constant values are in Hz and chemical shifts in δ.

*OCOMe: 1.92, 2.00, 2.01, 2.02, 2.07, 2.08, 2.09, 2.25, 2.26, 2.28, 2.34.

[a-e] Signals interchangeable. 6, 7 and 12 in $CDCl_3$-CD_3OD; 8 and 11 in $CDCl_3$.

Table 2. ^{13}C NMR chemical shift assignments of 6-8 and 11-12.[#]

	6	7	11*	12	8
C-1	76.2[a]	76.0[a]	74.1	76.7[a]	76.4[a]
C-2	83.0	82.6	80.1	83.0	74.3[a]
C-3	22.7	22.8	21.1	22.8[b]	24.2[b]
C-4	85.4	85.5	85.4	83.6[b]	83.4[b]
C-5	82.7	82.6	80.9	83.0[b]	83.3[b]
C-1'	132.3[b]	133.1[b]	134.4[a]	131.8	133.0
C-2'	116.0[b]	111.7[b]	122.3[a]	114.5[c]	114.5[c]
C-3'	145.7[c]	149.4	141.9[b]	145.3[d]	149.2[d]
C-4'	145.7[c]	149.4	141.9[b]	144.9[d]	149.0[d]
C-5'	119.9[d]	115.9	125.6[c]	118.8	114.0[c]
C-6'	119.9[d]	120.5	126.1[c]	119.7	119.2
C-1"	119.9[b]	118.5[b]	118.2[a]	115.2	114.8[e]
C-2"	115.2[b]	111.1[b]	123.1[a]	115.6[c]	111.3[f]
C-3"	145.8[c]	149.4	139.5[b]	145.4[d]	146.2[f]
C-4"	147.7[d]	147.0	147.9	144.9[d]	146.0[f]
C-5"	118.2[d]	117.0	114.4	115.6[c]	110.0[e]
C-6"	124.4	125.4	130.1	124.4	125.5
CO	168.4		165.1	168.2	
C-1"'	126.8		131.8	126.5	
C-2"'	130.7		129.4	130.5	
C-3"'	116.5		121.8	116.1	
C-4"'	160.3		151.9	159.9	
C-5"'	116.5		121.8	116.1	
C-6"'	130.7		129.4	130.5	
C-α	146.4		144.5	146.2	
C-β	114.8		116.9	114.7	
OMe		56.2 56.5			55.9
glucose					
C-1	101.3[e]	101.9[c]	101.7[d]	101.2	
C-2	75.8[e]	73.8[c]	71.3[e]	74.3	
C-3	74.3[e]	77.1[a]	72.5[f]	75.5[a]	
C-4	70.8	70.5[d]	68.5[f]	70.9	
C-5	77.0[a]	76.8[a]	72.5[e]	76.0[a]	
C-6	62.1	61.9	61.7[g]	61.9	
C-1'	103.7	102.8	97.4[d]		
C-2'	74.8	74.0[c]	70.5[d]		
C-3'	77.6[a]	77.1[a]	71.9[e]		
C-4'	71.2	70.4[d]	68.2[f]		
C-5'	77.6[a]	76.7[a]	71.7[e]		
C-6'	62.1	61.9	62.0[g]		

[#]Chemical shift values in ppm. [a-g] Signals interchangeable.
6,7 and 12 in $CDCl_3$-CD_3OD; 8 and 11 in $CDCl_3$
*OCOMe: 167.9, 168.7, 169.3, 169.4, 169.6, 170.0, 170.4, 170.6
OCOMe: 20.3, 20.6, 20.9, 21.1.

This compound, which gave positive reaction for phenols (Folin-Ciocalteu) showed the ^{1}H NMR signals, besides those of the three methoxy groups, of six aromatic hydrogens and an hydrogen at δ 4.66 , d, J=7.0 Hz, coupled with another at ca. 4.0 (partially overlapped) which is further coupled with a methylenic group (δ 2.59, dd, J=4.0 and 17.0 Hz, H_a, and 2.44, dd, J=6.0 and 17.0 Hz, H_b). The additional presence of a disubstituted acetylenic group (^{13}C NMR, 83.3 and 83.4 ppm, singlets in SFORD) suggested for 8 the structure of a trimethyl derivative of nyasicol, 9, the aglucone of nyasicoside, as confirmed by conversion of 8 into the known tetramethylnyasicol, 10, through methylation. Therefore only one of the two glucose units of interjectin is linked to a phenolic group, whereas the second is linked to the C-2 hydroxy group, since the adjacent hydrogen (δ 4.04, m) is not shifted downfield in the dodecaacetyl derivative of 6, 11, contrary to what occurs for H-1 (δ 6.10, d, J=6.0 Hz).

By hydrolysis of interjectin with β-glucosidase, only the glucose-phenol linkage was cleaved and the monoglucoside 12, $C_{32}H_{32}O_{13}$, was obtained. Its negative FAB mass spectrum - besides the ion at m/z 623 $[M-H]^-$ - showed the ion at m/z 459 corresponding to the loss of p-hydroxy cinnamic acid (164 m.u.).

In the ^{1}H NMR spectrum of 12, the signal of the anomeric hydrogen is a broad singlet (δ 4.93) overlapped with a peak of the doublet (δ 4.94, d, J=8.0 Hz) of the vicinal hydrogen. The former is namely the part A of a ABX system where the chemical shift values of A and B are very close. As the other glucose hydrogens, identified by spin decoupling (see table 1), have lower values, position 2 must be considered that acylated by p-hydroxy cinnamic acid. Like interjectin, also the monoglucoside 12 by methylation with diazomethane lost p-hydroxy cinnamic acid, and the resulting compound was identified as tetramethylnyasicoside, 13, by direct comparison.

By comparison of the ^{13}C NMR signals of the acetylenic moiety, the presence of two different values (ca. 82 and 85 ppm) was observed in 6, 7, and 11, whereas they are practically identical (ca. 83 ppm) in 8 and 12. This phenomenon observed for obtusides B and A by comparison with suitable models had allowed the assignment of glucose on C-4" on obtuside B, where the ^{13}C NMR values of the acetylenic carbons are different [6]. Analogously, in interjectin the glucose was assigned to C-4". The enzymatic hydrolysis with β-glucosidase and the values of the coupling constants of the two anomeric hydrogens (ca. 7 Hz) accounted for the β-glucosidic nature of the two glucose units. Since the absolute configuration of the two stereogenic centres C-1 and C-2 of nyasicoside had been unambiguously assigned [7], the structure of interjectin, 6, is

thus fully demonstrated.

The most polar fraction obtained by CCD of the extract of rhizomes was identified as a mixture of triglucosides of 5 in agreement with the isolation of 1,5-bis(3,4-dihydroxyphenyl)-1-penten-4-yne and glucose by hydrolysis, and the mass spectrum ($[M-H]^-$ at m/z 795) of the dimethyl derivatives.

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EXPERIMENTAL

A Craig Post apparatus (200 stages, 10:10 ml, upper and lower phase) was used for CCD. ^1H and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer, TMS as internal standard. ^1H and ^{13}C NMR data of compounds 6-8 and 11-12 are reported in Tables 1 and 2, respectively.

Plant material. Rhizomes of Hypoxis interjecta and H. multiceps (20-70 g each) were collected and identified by the Department of Agricultural Economics and Marketing, Pretoria, South Africa.

Extraction. Air dried rhizomes of H. interjecta (470 g) and H. multiceps (50 g) were exhaustively extracted with MeOH. The residues after evaporation of the solvent (56 g and 6 g, respectively) showed identical HPLC profile (Spherisorb 5 ODS reversed phase column, developed by gradient elution with solvent A, MeOH-H_2O (8:2), and solvent B, 0.05M phosphate buffer, pH 3.3, 10% up to 95% solvent A).

The extract was subjected by 5 g portions to CCD, using the biphase system H_2O:EtOAc:n-BuOH. With the ratio 10:9:1 obtusides A and B were eluted (K_r=3), whereas hypoxoside (K_r=0.9) and very near, interjectin (K_r=0.8), were eluted with the ratio 10:7.5:2.5, and, finally, a mixture of triglucosides of 5 (K_r=0.8) with the ratio 10:3:7.

Interjectin, 6. Crystals from EtOH, m.p. 156°C with dec.; $[\alpha]_D$=+1.0±0.5 (c 0.5, MeOH). UV (MeOH) λ_{max} nm: 315, 300, 291, 255 (log ε 4.60, 4.66, 4.69, 4.70). FAB MS (negative ion mode), m/z: 785 $[M-H]^-$, 623, 579, 275, 183; (positive ion mode), m/z: 769 $[M+H-H_2O]^+$, 461, 369, 277, 185. (Found: C, 57.85; H, 5.59. Calc. for $C_{38}H_{42}O_{18}$: C, 57.93; H, 5.50%).

Methylation of 6: 7. Interjectin, 6, dissolved in MeOH was methylated with ethereal solution of diazomethane. After two days the solvents were evaporated and the residue was purified by CCD with the solvent system H_2O:EtOAc:n-BuOH 10:7:3, K_r=0.16. The derivative, 7, crystallized from EtOH and EtOAc, m.p. 121-122°C, $[\alpha]_D$=-16.0 (c 1.2, MeOH); UV (MeOH) λ_{max}

nm: 295, 285, 255 (log ε 3.73, 3.84, 4.34). EI MS, M^+ at m/z 682, corresponding to $C_{32}H_{42}O_{16}$.

Hydrolysis of 7: 3',3",4'-trimethylnyasicol, 8. A solution of 7 (340 mg) in water (100 ml) and acetate buffer at pH 5.5 (20 ml) was submitted to hydrolysis with β-glucosidase (25 mg) at 34°C for 3 days. The turbid solution was then extracted with EtOAc and the residue of the organic phase was purified by CCD with the solvent system H_2O-acetone-cyclohexane-EtOAc, 6:4:5:3, K_r=0.4. The substance obtained, 8, m.p. 49-50°C from n-hexane, corresponding to the raw formula $C_{20}H_{22}O_6$, M^+ at m/z 358, gave positive reaction for phenols (Folin-Ciocalteu). The aqueous phase, after extraction with n-BuOH, was percolated through a column Dowex 50 W (H^+). In the residue D-glucose was identified by t.l.c. and through the corresponding β-pentaacetate by comparison with an authentic specimen of β-D-pentaacetylglucose.

Methylation of 8: tetramethylnyasicol, 10. By methylation with diazomethane 8 was made into tetramethylnyasicol, which was identified by direct comparison (^1H NMR data, rotatory power) [4].

Acetylation of 6: dodecaacetylinterjectin, 11. Interjectin was acetylated with a mixture (1:1) of pyridine and acetic anhydride. After two days the reagents were evaporated in vacuo at room temperature. The residue was purified by CCD with H_2O:acetone:cyclohexane, 4:5:8, K_r=0.2, mp 107-110 °C from n-hexane (Found: C, 57.50; H, 5.21. Calc. for $C_{62}H_{66}O_{30}$: C, 57.67; H, 5.15 %).

Partial hydrolysis of 6: 12. The hydrolysis was carried out as for 7. After 7 days part of the compound remained unaffected. The solution was then extracted with n-BuOH and the residue of the organic phase was purified by CCD with the solvent system H_2O-EtOAc-n-BuOH, 100:95:5. The obtained monoglucoside, K_r=2.5, amorphous powder, corresponds to the raw formula $C_{32}H_{32}O_{13}$, FAB MS (negative ion mode), m/z: 623 $[M-H]^-$, 551, 459, 367, 275, 183; (positive ion mode), m/z: 607 $[M+H-H_2O]^+$, 553, 461, 369, 277, 185.

Methylation of 12: tetramethylnyasicoside, 13. The monoglucoside 12 was methylated with diazomethane. The obtained compound was identified as tetramethylnyasicoside, 13, by direct comparison.

Hydrolysis of the triglucosides. The mixture of triglucosides was submitted to hydrolysis as reported for 7. After 3 days the reaction mixture was extracted with EtOAc and the residue of the organic phase was identified as 5 by direct comparison. In the aqueous phase D-glucose was identified as reported for the hydrolysis of 7.

Methylation of the triglucosides. The mixture of triglucosides dissolved in MeOH was methylated with diazomethane for two days. The FAB mass

spectrum (negative ion mode) gave account of the methylation of two hydroxy groups (795, $[M-H]^-$, and 309 $[aglucone-H]^-$).

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