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MONONYASINE A AND MONONYASINE B: TWO GLUCOSIDES FROM *HYPOXIS NYASICA**

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Abstract—From the rhizomes of *Hypoxis nyasica*, two monoglucosides having the same aglucones, nyasoside [1-(4′-hydroxyphenyl)-3-(4″-hydroxyphenyl)-1,4-pentadiene], were isolated The structures were assigned by comparison of their spectroscopic data (and of the corresponding methyl and tetrahydromethyl derivatives) with those of nyasoside (and tetrahydronyasoside).

INTRODUCTION

Hypoxis species are used in African traditional medicine for the treatment of urinary infections, prostatic hypertrophy and internal cancer [2, J. D. Msonthi, unpublished work]. The family of Hypoxidaceae, monocotyledons eventually distinct from Amaryllidaceae [3], includes other genera, such as *Curculigo*, *Pauridia*, *Spiloxene*, *Empodium*, *Rhodohypoxis*, *Campynema* and *Campynemathe*, spread throughout the southern hemisphere.

From the rhizomes of *Hypoxis nyasica* Bak of Malawi, we isolated three glucosides, hypoxoside (previously isolated from *Hypoxis obtusa* [4]), nyasoside (**1**) [5] and nyasicoside [6], whose aglucones having the skeleton Ph-C_5-Ph can be considered as being formed by coupling of two Ph-C_3 units, β-γ' in hypoxoside and nyasicoside and α-β' in nyasoside, with the simultaneous loss of the terminal carbon atom of one of the side chains. We now report on the isolation from the rhizomes of *H nyasica* of two new glucosides named mononyasine A (**2**) and mononyasine B (**3**).

RESULTS AND DISCUSSION

Compound **2** gave the molecular formula $C_{23}H_{26}O_7$ in agreement with the molecular ion peak at *m/z* 414 (3%) in its electron impact mass spectrum Its ^{1}H NMR spectrum shows the signals of two *para* disubstituted aromatic rings and those of a vinyl group (δ5 1–5 2, AB part, 6.05, X part) and a vinylene (δ5 70, *dd*, J = 10 and 11 Hz and 6.53, *d*, J = 11 Hz) whose signals at δ6.05 and 5.70 are further coupled with a hydrogen at δ4.56 (*dd*) A well differentiated doublet at δ4 93, J = 7 5 Hz, belongs to an anomeric hydrogen of a monose whose ^{13}C NMR signals are typical for a glucopyranoside (see Table 1).

Furthermore, **2**, which gives positive reactions for phenols (ferric–ferricyanide and Folin–Ciocalteus), gives a monomethyl derivative, **4**, on methylation with diazomethane (OMe, NMR· δ_H3 83, δ_C55.1) which in turn gives a tetrahydro derivative, **5** (MS, *m/z* 432, M^+ for $C_{24}H_{32}O_7$), on hydrogenation in the presence of 5% Pt ($BaSO_4$). The ^{13}C NMR data of **4** and **5** are reported in Table 1.

On hydrolysis with β-glucosidase, **2** gave D-glucose, confirmed through its β-pentacetate, and an oily aglucone which was identified as nyasol (**6**), the aglucone of nyasoside (**1**). Compound **2** is therefore a monoglucoside of nyasol

The ^{1}H and ^{13}C NMR data of the second compound isolated, **3**, are very similar to those of **2**. Also **3** gives nyasol and glucose on enzymatic hydrolysis and a monomethyl derivate, **7**, with diazomethane which is converted into the corresponding tetrahydro derivative, **8**, on catalytic hydrogenation.

The problem of the relationship between mononyasine A (**2**) and mononyasine B (**3**) and the two possible 4′ and 4″ monoglucosides of nyasol was solved by comparison of some diagnostic ^{13}C NMR signals with the corresponding ones of nyasoside and derivatives. Thus the signal of C-1″ in **1** (δ138 5, unambiguously assigned and well distinct from C-1′, δ133 1) is very near to that of mononyasine A (**2**) (δ138 9) and is markedly different from that of mononyasine B (**3**) (δ135.4). The same relationships hold for the corresponding methyl derivatives, **4** and **7**, where C-1″ resonates at δ137 7 and 135 8, respectively

Analogous correspondence is also observed on comparison of tetrahydronyasoside (**9**), tetrahydromethylmononyasine A (**5**) and tetrahydromethylmononyasine B (**8**) where C-1″ resonates at δ140.4, 140.1 and 137 5, respectively This behaviour is related to the different substitution in 4″ between **2** and **3** and therefore the glucosidic linkage is assigned to 4″ in **2** and to 4′ in **3**

Unlike C-1″, the resonance of C-1′ is practically unaffected by glucosylation at 4′ in the unsaturated compounds **1**–**4** and **7**, probably on account of the prominent effect of the conjugation with the 1-2 double bond. However, the same effect of glucosylation observed for C-1″ can be found for C-1′ in the corresponding tetrahydro derivatives, thus the resonance in **5** (δ135.2) is lower than in **8** and **9** (δ138.0 and 137 6, respectively).

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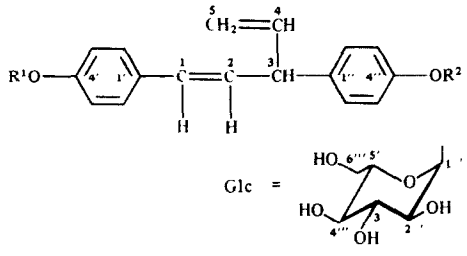
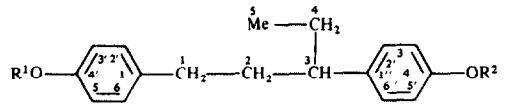


	R^1	R^2
1	Glc	Glc
2	H	Gl
3	Glc	H
4	Me	Glc
6	H	H
7	Glc	Me



	R^1	R^2
5	Me	Glc
8	Glc	Me
9	Glc	Glc

The structure **2**, 4″-β-D-glucopyranosylnyasol, for mononyasine A, and the structure **3**, 4′-β-D-glucopyranosylnyasol, for mononyasine B are thus unambiguously demonstrated Hinokiresinol, the *E* isomer of nyasol (disregarding the chirality at C-3), had been isolated from *Chamaecyparis obtusa* by Hirose *et al.* [7] but the subsequent papers of synthesis have introduced ambiguities between the two substances (and their methyl ethers). Thus the compound obtained by Beracierta and Whiting [8], identified as racemic dimethylhinokiresinol, is really racemic dimethylnyasol because of the comparable values for J_{1-2} (11–12 Hz). On the other hand, racemic dimethylhinokiresinol (J_{1-2} = 15 9 Hz) has been synthesized by Ameer *et al* [9], who, on account of the previous oversight, have wrongly assigned the configuration *Z* to dimethylhinokiresinol of Hirose *et al* [7] and wrongly identified it with dimethylnyasol, and, accordingly, hinokiresinol with nyasol (disregarding the chirality at C-3)

EXPERIMENTAL

A Craig Post apparatus (200 stages, 10·10 ml, upper and lower phase) was used for CCD and a QVD steady-state distribution apparatus (123 tubes, 10.10 ml) for DCCD TLC EtOAc–HOAc–H_2O (10 2 5, upper phase), anisaldehyde-sulphuric acid ^1H and ^{13}C NMR. 400 MHz and 100 MHz, respectively, CD_3OD–$CDCl_3$ (3 7), TMS as int standard β-Glucosidase from almonds was purchased from Fluka

Material. The rhizomes of *Hypoxis nyasica* collected in Malawi were re-planted in the Botanical Garden of the University La Sapienza, where a sample is kept

Extraction and separation The small rhizomes of the plant (640 g) were made to a mush and extracted with MeOH (×2) The residue of the combined extracts (105 g) was dissolved in H_2O (500 ml) and the soln was extracted with water saturated *n*-BuOH (3 × 200 ml) The residue of the organic phase (65 g) was submitted in portions to CCD with the solvent system H_2O–EtOAc–*n*-BuOH (4 3 1) and three fractions were obtained. one with $K_r > 6$ (3 4 g), the second, $K_r \simeq 1$ (39 g), which is mostly hypoxoside and nyasoside (**1**), and the third, $K_r = 0.35$, 7 g, so far not examined The more mobile, chromatographically uniform fraction was a mixture which was resolved by CCD on prolonged recycling (1800 transfers) and by DCCD with the biphasic system H_2O–Me_2CO–EtOAc–cyclohexane (20 11 18 8). The separation was monitored by NMR. The more mobile compound, K_r = 1 2, was named mononyasine A (**2**) whereas the less mobile compound, K_r = 1 1, was named mononyasine B (**3**)

Mononyasine A (**2**) Mp 169–170° from EtOAc, $[\alpha]_D^{20} = -166$ (MeOH, *c* 0.6); UV λ_{max}^{MeOH} nm 207, 257, 298 (sh) (log ε 4.40, 4 15, 3 43) Positive reaction with ferric–ferricyanide and Folin–Ciocalteus phenol reagents MS *m/z* (rel int) 414 $[M]^+$ (3), 252 (aglucone, 100), 237 (38), 158 (84), 145 (72), 107 (60), ^1H NMR, δ. 3 4–3 6 (4H, H-2–H-5 glc), 3.74 (1H, *dd*, *J* = 5 and 12 Hz, H_a-6 glc), 3.95 (1H, *dd*, *J* = 2 and 12 Hz, H_b-6 glc), 4 56 (1H, *dd*, *J* = 7 and 10 Hz, H-3), 4 93 (1H, *d*, *J* = 7 5 Hz, H-1 glc), 5 1–5.2 (2H, H_a-5 and H_b-5), 5 70 (1H, *dd*, *J* = 10 and 11 Hz, H-2), 6 05 (1H, *ddd*, *J* = 7, 10 and 16 Hz, H-4), 6 53 (1H, *d*, *J* = 11 Hz, H-1), 6 78 and 7 09 (2H and 2H, *d*, *J* = 8 Hz, H-3′, 5′, 3″, 5″), 7 15 and 7.18 (2H and 2H, *d*, *J* = 8 Hz, H-2′, 6′, 2″, –6″). (Found C, 66 98, H, 6.60, calcd for $C_{23}H_{26}O_7$ C, 66.65, H, 6.32%)

Mononyasine B (**3**) Mp 147–149° from EtOAc and *n*-hexane, $[\alpha]_D^{20} = -198$ (MeOH, *c* 0 6), MS *m/z* (rel int) 414 $[M]^+$ (5), 252 (100), ^1H NMR, δ 3 4–3.6 (4H, H-2–H-5 glc), 3 76 (1H, *dd*, *J* = 5 and 12 Hz, H_a-6 glc), 3 93 (1H, *dd*, *J* = 2 and 12 Hz, H_b-6 glc), 4 47 (1H, *dd*, *J* = 7 and 10 Hz, H-3), 4 95 (1H, *d*, *J* = 7 5 Hz, H-1 glc), 5 1–5 2 (2H, H_a-5 and H_b-5), 5 72 (1H, *dd*, *J* = 10 and 11 Hz, H-2), 6 00 (1H, *ddd*, *J* = 7, 10 and 16 Hz, H-4), 6 52 (1H, *d*, *J* = 11 Hz, H-1), 6 76 and 7 06 (2H and 2H, *d*, *J* = 8 Hz, H-3′, 5, 3″, 5″), 7 06 and 7 23 (2H and 2H, *d*, *J* = 8 Hz, H-2′, 6′, 2″, 6″)

Methylmononyasine A(**4**) Amorphous powder, $[\alpha]_D^{20} = -149$ (MeOH, *c* 0 6), obtained by methylation of **2** in MeOH with ethereal CH_2N_2 and subsequent purification by CCD with solvent system H_2O–EtOH–EtOAc–cyclohexane (5 2 3 4), K_r = 1 5, ^1H NMR, δ 3 4–3 6 (H-4, H-2–H-5 glc), 3 78 and 3 90 (H_a and H_b-6 glc), 3 83 (OMe), 4.5 (H-3 obscured by HDO), 4 91 (H-1 glc), 5 1-5.2 (H_2-5), 5 60 (H-2), 6 03 (H-4), 6 54 (H-1), 6.90 and 7 04 (H-3′, 5′, 3″, 5″), 7 18 and 7 23 (H-2′, 6′, 2″, 6″)

Tetrahydromethylmononyasine A (**5**) Amorphous powder obtained by catalytic hydrogenation of **4** with 5% Pt ($BaSO_4$) in 90% aq MeOH MS, *m/z* (rel int) 432 $[M]^+$ ($C_{24}H_{32}O_7$, 21), 270 (aglucone, 100), ^1H NMR, δ 0 75 (3H, *t*, *J* = 7, H_3-5), 1 5–2 0 (4H, H_2-2 and H_2-4), 2 35–2 45 (3H, H_2-1 and H-3), 3 4–3 6 (4H, H-2–H-5 glc), 3 80 (OMe), 3 90 (2H, H_2-6 glc), 4.95 (1H, *d*, *J* = 7 5 Hz, H-1 glc), 6 8–7 1 (8H arom)

Hydrolysis of mononyasine A nyasol (**6**) β-Glucosidase (5 mg) was added to a soln of mononyasine A (50 mg) in acetate buffer, pH 5 5 (20 ml). The soln was covered by toluene and allowed to stand at 36° overnight After addition of a few drops of HOAc, the aq phase was extracted with EtOAc The residue of the organic phase was purified by CCD with the solvent system H_2O–Me_2CO–cyclohexane–EtOAc (5 5 5 1) The oily aglucone was identified as nyasol (**6**) by comparison of the NMR data

Table 1. ^{13}C NMR spectral data of compounds **1–5** and **7–9**

C	2	3	4	5	7	8	1	9
1	132 1	133 5	131 4	33.2	132 7	33.3	132 5	33.6
2	129.9	129 1	128.4	38 8	128.2	38.8	130.7	39 1
3	48 4	48 0	46 8	46 9	46.7	46.8	48.1	47 4
4	142.3	142 2	140 5	30 2	141.0	30.1	142 1	30 5
5	115 1	114 9	114.9	12 1	114.8	12 1	115.2	12.2
1′	132 8	132.7	132.4	135 2	132.1	138.0[d]	133.1	137 6
2′,6′	130 8[a]	130.6[a]	129.6[a]	129 5[a]	129.6[a]	129.6[a]	130.7[a]	129 8[a]
3′,5′	116 0[b]	117.4[b]	113 6[b]	114 1[b]	116.3[b]	117 0[b]	117 4[b]	117 6[b]
4′	157 5	157.0	155 7	156.0	158 1	158.2	157 9[c]	156 8[c]
1″	138 9	135 4	137 7	140.1	135 8	137.5[d]	138 5	140 4
2″,6″	129 6[a]	129.4[a]	128 6[a]	129.0[a]	128.7[a]	129.0[a]	129 5[a]	129 4[a]
3″,5″	118.0[b]	116 2[b]	116 7[b]	116.9[b]	114.8[b]	114 1[b]	118.0[b]	117.5[b]
4″	157 5	157 0	158.5	158.0	156 7	155 8	157.5[c]	156.6[c]
OMe			55.1	55.5	55.5	55 5		
1‴	102 4	102.1	100 9	101.6	100 7	101.6	102.5	102.3, 102.2
2‴	74 9	74.7	73.3	73 9	73.3	73.9	74.9	74 5
3‴	78.0	77 8	76.3[c]	77 0[c]	76.3[c]	77.0[c]	77 8[c]	77.6[c]
4‴	71 4	71.3	69 9	70.5	69.9	70.5	71 4	71.1
5‴	78 0	77 8	76.0[c]	76.7[c]	76.0[c]	76.7[c]	77 9[c]	77.4[c]
6‴	62 5	62 5	61.7	62.1	61 7	62 1	62.5	62.4

[a–c] These values may be interchanged in the same column.

and the specific optical rotation [6]. The aq soln was extracted with *n*-BuOH and then percolated through a column of Dowex 50 W (H^+) In the residue, glucose was identified by TLC (H_2O–MeOH–HOAc–CH_2Cl_2 2:3·5 10) and through its β-pentacetate by comparison with an authentic specimen.

Methylmononyasine B (**7**). Amorphous powder, $[\alpha]_D^{20} = -177$ (MeOH; *c* 0.8), obtained by methylation of **3** as reported for **4** ^1H NMR, δ 3.4–3.6 (4H, H-2–H-5 glc), 3 79 (OMe), 3.92 (H_a-6 glc, H_b obscured), 4.48 (H-3), 4.94 (H-1 glc), 5.1–5 2 (H_2-5), 5.72 (H-2), 6.00 (H-4), 6 52 (H-1), 6 85 and 7.04 (H-3′, 5′, 3″, 5″), 7.14 and 7 22 (H-2′, 6′, 2″, 6″)

Tetrahydromethylmononyasine B (**8**). Amorphous powder obtained by catalytic hydrogenation of **7** with 5% Pt ($BaSO_4$) in 90%aq. MeOH ^1H NMR, δ. 0 77 (H_3-5), 1.5–2.0 (H_2-2 and H_2-4), 2.3–2 4 (H_2-1 and H-3), 3.4–3 6 (4H, H-2–H-5 glc), 3.77 and 3 89 (H_2-6 glc), 3.82 (OMe), 4 89 (H-1 glc), 6 8–7 1 (8-H arom)

Hydrolysis of mononyasine B was performed as described for mononyasine A, to give D-glucose and nyasol.

Tetrahydronyasoside (**9**). Mp 118–121° from EtOAc; $[\alpha]_D^{20} = -69$ (MeOH; *c* 0.6) obtained by catalytic hydrogenation of nyasoside (**1**) with 5% Pt ($BaSO_4$) in 90% aq. MeOH. FAB MS, *m/z*: 580 $[M]^+$, 418 $[M-glc]^+$, 256 (aglucone), ^1H NMR: δ0 75 (H_3-5), 1 5–2 0 (H_2-2 and H_2-4), 2.3–2.4 (H_2-1 and H-3), 3 4–3.6 (8H, H-2–H-5, two glc), 3.7–4.0 (4H, H_2-6, two glc), 4.85 and 4 91 (2H, H-1, two glc), 6 98 and 7 05 (4H and 4H arom)

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