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4-Oxonicotinamide-1-(1′-β-D-ribofuranoside) from *Rothmannia longiflora* Salisb. (Rubiaceae)

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Abstract

The first isolation of 4-oxonicotinamide-1-(1′-β-D-ribofuranoside) from a nonmammalian source, the West African plant *Rothmannia longiflora* Salisb. (Rubiaceae), is described. Its stereostructure was established by spectroscopic methods and an X-ray structure analysis of its tri-*O*-acetyl derivative. This *N*-glycoside had previously been isolated only from human urine. In addition, the plant was found to contain monomethyl fumarate and D-mannitol.

Keywords: *Rothmannia longiflora*; Rubiaceae; Structural elucidation; X-ray crystallography; NMR GIAO calculations; 4-Oxonicotinamide-1-(1′-β-D-ribofuranoside); 4-Oxonicotinamide-1-(1′-β-D-2′,3′,5′-tri-*O*-acetylribofuranoside); Fumaric acid monomethylester; D-Mannitol

1. Introduction

Rothmannia longiflora Salisb. (Rubiaceae) is a shrub or tree which is widespread in tropical Africa (Hutchinson, & Dalziel, 1963). Aqueous extracts and the powdered leaves are used in the traditional medicine of the West African Oubis people for the treatment of diarrhoea (Adjanohoun, & Aké Assi, 1979). Furthermore, applications as analgesic, emetic and fever remedy have been described (Abbiw, 1990). The related South African plant species *R. globosa* contains several iridoids (Jensen, 1983) and from *R. hispida* a not yet fully characterized dye was isolated (Ashraf, Ekpenyong, Nair, Akpan, & Kpagh, 1990), but no phytochemical studies have to date been performed on *R. longiflora*. In this paper we describe the isolation of the unusual *N*-glycoside 4-oxonicotinamide-1-(1′-β-D-ribofuranoside) (**1**)[1], its structural elucidation by spectroscopic methods, and the confirmation by an X-ray structure analysis of its triacetyl derivative. In addition to **1** we have identified the widespread natural products D-mannitol and fumaric acid monomethyl ester in *R. longiflora*.

2. Results and discussion

A methanolic extract of the dried and powdered fruits of *R. longiflora* was subjected to consecutive separation on polyamide gel, Sephadex LH-20, and finally Lobar® RP-18, to afford crystalline D-mannitol, identical to an authentic sample by its spectral (IR,

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[1] An alternative name for **1**, as also used in Wolinski, Hilton, and Pulay (1990) and Erlenmeyer, and Schoenauer (1937), is 1-β-D-ribofuranosylpyridin-4-one-3-carboxamide.

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NMR, MS) data and a nitrogen-containing solid compound.

Electrospray ionization mass spectrometry of the solid substance in the positive mode yielded molecular ions $[M + H]^+$, *m/z* 271 and $[M + Na]^+$, *m/z* 293, indicating a molecular mass of 270 amu. MS/MS-experiments focusing on precursor ions *m/z* 271 and *m/z* 293 revealed product ions *m/z* 139 and *m/z* 161, due to the neutral loss of 132 amu. Thus, the isolated natural product was a glycoside consisting of a pentose (132 amu) and the corresponding aglycone (138 amu). The ions *m/z* 139 $[\text{aglycone} + H]^+$ and *m/z* 133 $[\text{pentose moiety} + H]^+$, but no molecular ion was detected by CI conditions with isobutane as the reagent gas.

High resolution mass spectrometry using EI of *m/z* 138 yielded a molecular formula corresponding to $C_6H_6N_2O_2$ for the aglycone unit. Further evidence of the structure of the aglycone were the ions $[C_6H_4NO_2]^+$, *m/z* 122 and $[C_5H_4NO]^+$, *m/z* 94, which were indicative of an amide group.

The ^1H NMR spectrum showed resonances of an aromatic ring and a pentose. The coupling pattern and the marked downfield shift of three aromatic protons were typical of a 3,4-bisubstituted aromatic ring containing one nitrogen. The HMBC and NOE spectra (see Fig. 1) confirmed this and showed the amide group to be located at C-3 and the sugar to be connected with the ring nitrogen of the aglycone at position C-1′. The assignment of the ^{13}C signals in the

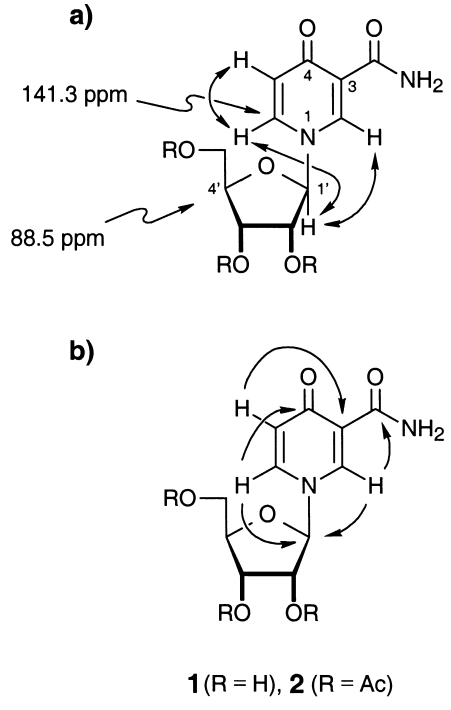

Fig. 1. Structure of the glycoside **1** and its tri-*O*-acetylated derivative **2**, by selected ^{13}C NMR chemical shifts and NOE effects (a) and by HMBC interactions (b).

Table 1
Experimental (a) and calculated (b) ^{13}C NMR chemical shifts (*δ*-values in ppm) of **1**

Atom	a	b
C-5′	63.36	62.2
C-3′	72.50	65.4
C-2′	78.10	73.2
C-4′	88.53	82.8
C-1′	99.47	96.8
C-3	120.81	113.5
C-5	122.86	119.3
C-6	141.29	140.2
C-2	145.26	160.4
$CONH_2$	170.35	173.9
C-4	181.72	181.3

aromatic ring was achieved by HMQC and HMBC experiments.

The downfield shift (88.5 ppm) of C-4′ in ^{13}C NMR revealed that the sugar must be a furanose (Bock, & Pedersen, 1983). The ^{13}C NMR signals of the sugar were again assigned with the help of HMQC and HMBC experiments. The ribose unit was identified by a cleavage experiment under acidic conditions and comparison of the pentose formed by TLC with an authentic reference of D-ribose. The ^1H NMR spectra of the compound were all in agreement with structure 1 and matched with the 200 MHz data given for 4-oxonicotinamide-1-(1′-β-D-ribofuranoside) (**1**) in Dutta, Drain, McCloskey, and Chheda (1979), Srikrishnan, Parthasarathy, Alderfer, Dutta, and Chheda (1988) and Dutta, and Chheda (1991). Since ^{13}C NMR data for this compound were not given in those publications, they are presented in this paper. Furthermore, quantumchemical calculations of the ^{13}C NMR data of **1** were carried out, starting from an input geometry preoptimized with the TRIPOS force field. The conformational analysis was done by semi-empirical AM1 calculations, and the minimum structure found was refined by an ab initio RHF/3-21G geometry optimization, followed by ^{13}C NMR calculations using the GIAO method (Wolinski et al., 1990). The calculated values (see Table 1) show good agreement with the experimental data and thus confirm the attribution of the signals.

Compound **1** was derivatized by peracetylation of the sugar moiety. The resulting triacetate **2** gave crystals suitable for an X-ray structure analysis (see Fig. 2), which confirmed the constitution and revealed that the glycoside has β-configuration.

According to TLC both compounds, **1** and D-mannitol, are also present in the methanolic extract of the leaves and branches. From this extract the *N*-glycoside **1** was isolated by centrifugal TLC.

As an alternative to the neutral methanolic extraction procedure of the fruits described above, the leaves

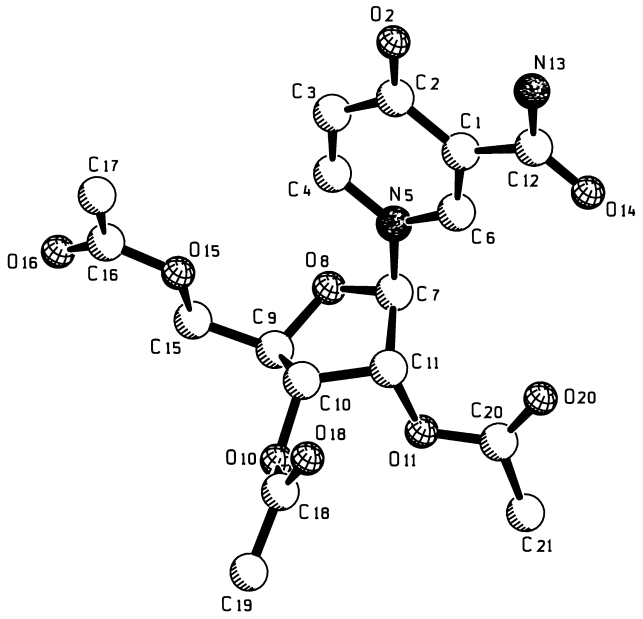

Fig. 2. The stereostructure of **2** in the crystal; hydrogen atoms have been omitted for reasons of clarity.

and branches were macerated with methanol–water–0.9 M HOAc. After evaporation of the methanol the aqueous phase was extracted consecutively with *n*-hexane and then chloroform. The chloroform extract was resolved by high speed countercurrent chromatography (HSCCC), with methanol–water–0.9 M HOAc as the eluent to afford monomethyl fumarate as a colorless crystalline compound. This monoester was identified by its spectroscopic data (MS, NMR, IR), which were identical to those of a reference obtained from fumaric acid dimethylester by partial hydrolysis according to Erlenmeyer, and Schoenauer (1937). TLC comparison of the genuine extracts, however, indicated that monomethyl fumarate was absent in material gained by neutral extraction. This suggests that the ester is a derivative of an as yet unknown natural product as a result of the acidic conditions. A search for the genuine source of the ester is in progress. Monomethyl fumarate has been found previously in the higher plant species *Tagetes minuta* (Compositae) (Ickes, Fong, Schiff, Perdue, & Farnsworth, 1973) and *Cudrania javanensis* (Moraceae) (Knapp, & Schiff, 1971). In the latter case, the compound was likewise isolated under acidic conditions and thus might have been a workup artifact, too.

Of the three (biosynthetically unrelated) natural products from *R. longiflora* presented in this paper, D-mannitol is widespread in higher plants. By contrast, monomethyl fumarate as a natural product is rare, whereas fumaric acid itself as a primary metabolite is common to higher plants. The *N*-glycoside **1** has so far been isolated from human urine, exclusively (Dutta et al., 1979; Mills, & Davis, 1989), never from higher plants.

3. Experimental

3.1. General

Mps uncorr. Optical rotations: 10 cm cell, MeOH, $CHCl_3$. IR: KBr. ^1H NMR (250 or 600 MHz, Bruker) and ^{13}C NMR (62.9 and 150.9 MHz, Bruker) were recorded in D_2O (external calibration), CD_3OD (solvent as internal standard, δ 3.30 and δ 49.02, resp.), $CDCl_3$ (solvent as internal standard, δ 7.26 and δ 77.01 resp.), DMSO-d_6 (solvent as internal standard, δ 2.50). Proton detected, heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC} = 150$ Hz) and HMBC (optimized for $^nJ_{HC} = 7$ Hz). The force field and the semiempirical AM1 calculations were carried out on Silicon Graphics IRIS 4D/310 GTX and INDIGO (R4000) workstations using the SYBYL program package[2] and the VAMP 6.1 program (Rauhut et al.), respectively. The ab initio calculations were performed on a Fujitsu VPP700 supercomputer at the Leibniz Rechenzentrum in Munich using the Gaussian 94 program package (Frisch et al., 1995). EIMS: 70 eV. CIMS: isobutane as reagent gas, electron energy 70 eV, source pressure held at 0.3 mbar and temp. at 130–140°. MS/MS analysis: performed on a triple stage quadrupole TSQ 7000 MS/MS system with electrospray interface (ESI) (Finnigan MAT), temp. of the heated capillary 200°C. ESI voltage set to 3.0 kV. N_2 served both as sheath (50 psi) and auxiliary gas. Runs were performed by loop injection in positive mode. CC: polyamide (50–160 mesh, Machery und Nagel), Sephadex LH-20 (Pharmacia), Lobar® RP-18 (Size B, Merck). TLC: precoated silica gel 60 F_{254} plates (Merck), Cellulose (Merck), spots were detected under UV light and stained by methanolic H_2SO_4 with subsequent heating. Chromatotron (Harrison Research): silica gel 60 PF_{254} (containing gypsum, Merck). HSCCC: $CHCl_3$–MeOH–0.1 M HOAc (5:5:3), mobile phase: lower phase, (H)→T, Triple Coil No. 14, 1.7×950 mm (large coil), TLC detection (see above), flow 2.5 ml min^{-1}, 900 min^{-1} (Conway, 1990). The X-ray data were obtained at room temp. on a Siemens P4 diffractometer using graphite monochromatized MoKα radiation.

3.2. Plant material

Fruits, branches and leaves from a specimen of *R. longiflora* Salisb. were collected in Subri Forest

[2] SYBYL: Tripos Inc., 1699 S. Hanley Road, St. Louis, MO, 63144-2913, USA.

Reserve, Daboase, South West Ghana (F.S.K.T.) and in Ivory Coast (L.A.A.). Additional plant material was purchased from A.A. Enti (Forestry Enterprises, Accra, Ghana). A voucher specimen (No. 33) has been deposited in Herbarium Bringmann.

3.3. Extraction and isolation

The dried fruits (50.0 g) and a mixture of the branches and leaves (92.0 g) were treated separately. The plant material was milled and extracted subsequently with petrol, CH_2Cl_2, and MeOH in a Soxhlet extractor. In a third extraction the branches and leaves (120 g) were macerated with MeOH–H_2O–0.9 M HOAc (5:1:1). After evapn of MeOH, the aq. soln was extracted with *n*-hexane and $CHCl_3$ and the solvents were evapd in vacuo.

3.4. Isolation of D-mannitol

0.50 g of the crude methanolic fruit extract were fractionated on polyamide (MeOH–H_2O, 1:1 → 1:0) and Sephadex LH-20 (MeOH–H_2O, 3:1). A viscous mass was obtained, from which D-mannitol gave colorless crystals (76 mg). Mp 162–164° (MeOH–H_2O); (The Merck Index, 1989) mp 166–168° (MeOH). $[\alpha]_D^{20}$ +22° (H_2O–1% borax, *c* 8.0); (The Merck Index, 1989) $[\alpha]_D^{20}$ +23–24° (H_2O–13% borax, *c* 10.0). The physical and spectroscopic (IR, NMR, MS) data were identical to those of an authentic sample obtained from Aldrich. By TLC, D-mannitol was also detected in the crude methanolic extract of branches and leaves.

3.5. Isolation of 4-oxonicotinamide-1-(1′-β-D-ribofuranoside) (*1*)

The mother liquor obtained by crystallization of D-mannitol (see above) was fractionated on Lobar® RP-18 with MeOH–H_2O (1:3) as the eluent at a pressure of 3 bar (3 ml/min) to give 1 (7.5 mg). An alternative isolation method proved to be more economical: 100 mg of the MeOH-extract of the branches and leaves were purified two times by centrifugal TLC using 4 mm plates and eluting with CH_2Cl_2–MeOH (10:0 → 6:4). Final precipitation from MeOH afforded 1 (13.3 mg) as a colorless microcrystalline powder. Mp 205–206° (EtOH); (Dutta et al., 1979; Srikrishnan et al., 1988; Dutta, & Chheda, 1991) mp 211–212° (EtOH). $[\alpha]_D^{25}$ −97.1° (MeOH; *c* 0.25); (Dutta et al., 1979; Srikrishnan et al., 1988; Mills, & Davis, 1989; Dutta, & Chheda, 1991) $[\alpha]_D$ not given. IR ν_{max} cm^{-1}: 3210 (O–H), 1650 (C=O), 1530 (C=O), 1180, 1110, 1030. ^1H NMR (600 MHz, D_2O): δ 3.78 (1H, dd, J_{gem} = 12.8 Hz, J_{vic} = 4.1 Hz, H-5′), 3.86 (1H, dd, J_{gem} = 12.8 Hz, J_{vic} = 3.1 Hz, H-5′), 4.20 (1H, dd, J = 3.9 Hz, J = 3.3 Hz, H-4′), 4.23 (1H, dd, J = 5.2 Hz, J = 3.7 Hz, H-3′), 4.29 (1H, dd, J = 5.4 Hz, J = 5.4 Hz, H-2′), 5.61 (1H, d, J = 5.6 Hz, H-1′), 6.67 (1H, d, J = 7.6 Hz, H-5), 8.00 (1H, dd, J = 7.8 Hz, J = 2.3 Hz, H-6), 8.75 (1H, d, J = 2.5 Hz, H-2). ^1H NMR (600 MHz, DMSO-d_6): δ 3.59–3.66 (2H, m, H-5′), 3.99–4.05 (3H, m, overlapped, H-2′, H-3′, H-4′), 5.16 (1H, t, J = 5.0 Hz, J = 5.0 Hz, 5′-OH) 5.25 (1H, d, J = 4.2 Hz, 2′-OH or 3′-OH), 5.50 (1H, d, J = 6.1 Hz, 1′-H), 5.52 (1H, d, J = 6.5 Hz, 2′-OH or 3′-OH), 6.47 (1H, d, J = 7.6 Hz, H-5), 7.49 (1H, d, J = 4.9 Hz, NH), 8.07 (1H, d, J = 7.6 Hz, J = 2.4 Hz, H-6), 8.65 (1H, d, J = 2.4 Hz, H-2), 9.45 (1H, d, J = 4.9 Hz, NH). ^{13}C NMR (150 MHz, D_2O): δ 63.36 (C-5′), 72.50 (C-3′), 78.10 (C-2′), 88.53 (C-4′), 99.47 (C-1′), 120.81 (C-3), 122.86 (C-5), 141.29 (C-6), 145.26 (C-2), 170.35 ($CONH_2$), 181.72 (C-4). The ^{13}C attributions were achieved by HMQC and HMBC experiments. EIMS *m/z* (rel. int.): 138 [M–$C_5H_8O_4$]+ (100), 122 [M–$C_5H_8O_4$–NH_2]$^+$ (19), 121 [M–$C_5H_8O_4$–NH_3]$^+$ (45), 94 [M–$C_5H_8O_4$–$CONH_2$]$^+$ (30), 93 [M–$C_5H_8O_4$–$CONH_3$]$^+$ (25). HREIMS *m/z*: 138.043 [M–$C_5H_8O_4$]$^+$ ($C_6H_6N_2O_2$ requires 138.043), 122.023 [M–$C_5H_8O_4$–NH_2]$^+$ ($C_6H_4NO_2$ requires 122.024), 121.016 [M–$C_5H_8O_4$–NH_2–H]$^+$ ($C_6H_3NO_2$ requires 121.016), 94.029 [M–$C_5H_8O_4$–$CONH_2$]$^+$ (C_5H_4NO requires 94.029), 93.022 [M–$C_5H_8O_4$–$CONH_2$–H]$^+$ (C_5H_3NO requires 93.021). ESI-MS *m/z* (positive mode): 293 [M + Na]$^+$, 271 [M + H]$^+$. MS/MS (coll. an. 15 eV, 2.0 mTorr Ar, scanning product ions 200–300 *m/z* in 3 s): *m/z* 293 [M + Na]$^+$: 161 [M–$C_5H_8O_4$ + Na]$^+$, *m/z* 271 [M + H]$^+$: 139 [M–$C_5H_8O_4$ + H]$^+$. CIMS (isobutane) *m/z* (rel. int.): 139 [M–$C_5H_8O_4$ + H]$^+$ (100), 133 [M–$C_6H_6N_2O_2$ + H]$^+$ (23).

3.6. Conformational analysis and NMR calculations on *1*

The preoptimized input geometries for the AM1 calculations were generated by the TRIPOS force field within the SYBYL program package. The conformational space of **1** was searched semiempirically by AM1 calculation of reaction coordinates of the flexible dihedral angles using the corresponding keywords of the VAMP program package (Rauhut et al.). Geometry optimizations were done by applying the EF algorithm with a gradient norm specification of 0.1 mdyn/Å. The global minimum structure was chosen for further ab initio calculations. The RHF/3-21G minimized geometry structure was determined using the default optimization algorithm as implemented in Gaussian 94 (Frisch et al., 1995). Based on this structure, the ^{13}C NMR properties were calculated with the GIAO method (Wolinski et al., 1990; Frisch et al., 1995) (see Tables 1 and 2).

3.7. Acid cleavage of **1**

Glycoside **1** (1 mg) in 1 ml 2 N HCl–H_2O–MeOH (4:1:5) was heated on a steam bath overnight and neutralized with 2 N NH_3 and evapd to dryness. The sugar sample was directly analyzed by TLC on cellulose (double run) using the solvent system *n*-BuOH–pyridine–HOAc–EtOAc–H_2O (10:4:2:5:4) and visualized with aniline phthalic acid reagent after heating at 110° for 5 min (El Sayed et al., 1995). It proved to be identical with an authentic sample obtained from Merck.

3.8. Acetylation of **1**

To 11.6 mg of **1** in 1 ml pyridine 1 ml Ac_2O was added. The reaction mixt. was stirred overnight at room temp. The soln was then concd to dryness in vacuo and purified by chromatography on a silica gel column eluted with CH_2Cl_2–MeOH (100:6). The product, 4-oxonicotinamide-1-(1′-β-D-2′,3′,5′-tri-*O*-acetylribofuranoside (**2**), was obtained as colorless needles (14.0 mg) from EtOAc–CH_2Cl_2–*n*-pentane. Mp 198–201°. $[\alpha]_D^{25}$ −107.6° ($CHCl_3$; *c* 0.26). IR ν_{max} cm^{-1}: 3420, 1730 (C=O), 1660 (C=O), 1540 (C=O), 1230, 1220, 1210. ^1H NMR (600 MHz, $CDCl_3$): δ 2.10 (3H, s, Ac), 2.14 (3H, s, Ac), 2.22 (3H, s, Ac), 4.36 (1H, dd, J_{gem} = 12.5 Hz, J_{vic} = 2.3 Hz, H-5′), 4.45 (1H, dd, J_{gem} = 12.5 Hz, J_{vic} = 2.8 Hz, H-5′), 5.25 (1H, dd, J = 11.0 Hz, J = 5.2 Hz, H-2′), 5.33 (1H, dd, J = 5.4 Hz, J = 3.6 Hz, H-4′), 5.45 (1H, dd, J = 11.1 Hz, J = 5.6 Hz, H-3′), 5.58 (1H, d, J = 5.9 Hz, H-1′), 6.06 (1H, s, NH), 6.63 (1H, d, J = 7.7 Hz, H-5), 7.60 (1H, dd, J = 7.7 Hz, J = 2.3 Hz, H-6), 8.72 (1H, d, J = 2.5 Hz, H-2), 9.79 (1H, s, NH). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 20.29 (Ac), 20.45 (Ac), 20.67 (Ac), 62.74 (C-5′), 70.24 (C-4′), 74.40 (C-2′), 94.56 (C-1′), 102.90 (C-3′), 119.60 (C-3), 121.56 (C-5), 135.83 (C-6), 141.86 (C-2), 165.95 ($CONH_2$), 169.34 (Ac), 169.50 (Ac), 170.37 (Ac), 178.34 (C-4). The ^{13}C attributions were achieved by HMQC and HMBC experiments. EIMS *m/z* (rel.int.): 396 $[M]^+$ (12), 259 $[M–C_6H_5N_2O_2]^+$ (100), 139 $[M–C_{11}H_{15}O_7 + 1]^+$ (90), 43 $[Ac]^+$ (95). HREIMS *m/z*: 396.117 $[M]^+$ ($C_{17}H_{20}N_2O_9$ requires 396.117).

3.9. X-ray structure analysis of compound **2**

Crystals suited for an X-ray structure analysis were obtained from MeOH. Crystal dimensions, 0.2 × 0.3 × 2.3 mm, $C_{17}H_{20}N_2O_9$, M_r = 396.35, orthorhombic, a = 8.218(2), b = 13.783(2), c = 16.303(3) Å, V = 1846.7(7) $Å^3$, space group $P2_12_12_1$, #19, Z = 4, D_c = 1.426 g cm^{-3}, μ(MoKα) = 1.2 cm^{-1}, F(000) = 832. The final refinement converged to R = 0.056 and R_W = 0.051. Atomic coordinates, bond lengths and angles and thermal parameters may be obtained from the Cambridge Crystallographic Data Centre on quoting the depository number CCDC 109904.

Table 2
Atomic parameters ($\times 10^4$) and equivalent isotropic displacement parameters ($pm^2 \times 10^{-1}$) of nonhydrogen atoms for compound **2**

Atom	*x*	*y*	*z*	U_{eq}
C(1)	3159(4)	7538(2)	7019(2)	41(1)
C(2)	1920(4)	7707(2)	7644(2)	52(1)
O(2)	2226(3)	8029(2)	8346(1)	80(1)
C(3)	305(4)	7484(3)	7393(2)	56(1)
C(4)	−50(4)	7128(2)	6646(2)	49(1)
N(5)	1152(3)	6950(2)	6092(1)	39(1)
C(6)	2706(3)	7161(2)	6279(2)	40(1)
C(7)	776(4)	6528(2)	5260(2)	43(1)
O(8)	−900(3)	6473(1)	5163(1)	46(1)
C(9)	−1457(4)	5480(2)	5143(2)	44(1)
C(10)	−16(4)	4915(2)	5469(2)	42(1)
O(10)	−123(3)	3940(1)	5149(1)	47(1)
C(11)	1438(4)	5491(2)	5194(2)	43(1)
O(11)	1712(3)	5253(2)	4342(1)	48(1)
C(12)	4937(4)	7715(2)	7144(2)	47(1)
N(13)	5320(4)	8004(3)	7901(2)	70(1)
O(14)	5924(3)	7583(2)	6596(1)	62(1)
C(15)	−3020(4)	5373(2)	5602(2)	52(1)
O(15)	−2686(3)	5438(2)	6465(1)	54(1)
C(16)	−4011(4)	5362(2)	6947(2)	57(1)
O(16)	−5349(3)	5263(2)	6695(2)	76(1)
C(17)	−3494(6)	5418(3)	7838(2)	81(2)
C(18)	830(4)	3284(2)	5516(2)	50(1)
O(18)	1792(3)	3489(2)	6031(1)	63(1)
C(19)	572(5)	2291(3)	5181(2)	75(1)
C(20)	3153(4)	5514(3)	4033(2)	53(1)
O(20)	4126(3)	5990(2)	4406(1)	75(1)
C(21)	3349(5)	5155(3)	3166(2)	70(1)

3.10. Isolation of fumaric acid monomethylester

The evapn residue of the $CHCl_3$ extract of the leaves and branches was separated by HSCCC giving fumaric acid monomethylester (13.0 mg) as colorless crystals, mp 142° (MeOH–H_2O); (Erlenmeyer, & Schoenauer, 1937) mp 143°. The spectroscopic data (IR, NMR, MS) were identical to those of an authentic sample synthesized according to Erlenmeyer, and Schoenauer (1937).

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References

Abbiw, D. (1990). *Useful plants of Ghana*. Kew: Intermediate Technology Publications and The Royal Botanical Gardens.

Adjanohoun, É., & Aké Assi, L. (1979). Contribution au récencement des plantes médicinales de Côte d'Ivoire (pp. 264). Centre Nationale de Floristique, Abidjan: Université d'Abidjan.

Ashraf, C. M., Ekpenyong, K. I., Nair, M. T. S., Akpan, N. B., & Kpagh, A. K. (1990). *Journal of the Chemical Society of Pakistan,*, *12,* 67 (Chemical Abstracts, 1990, 113, 193441c).

Bock, K., & Pedersen, C. (1983). In: R. S. Tipson, & D. Horton . Advances in carbohydrate chemistry and biochemistry, Vol. 41 (pp. 27). New York: Academic Press.

Conway, W. D. (1990). *Countercurrent chromatography*. New York: VCH Cambridge.

Dutta, S. P., & Chheda, G. B. (1991). *Nucleic Acid Chemistry*, *4,* 152.

Dutta, S. P., Drain, P. F., McCloskey, J. A., & Chheda, G. B. (1979). *Life Science*, *24,* 1381.

El Sayed, K. A., Halim, A. F., Zaghloul, A. M., McChesney, J. D., Stone, M. P., Voehler, M., & Hayashi, K. (1995). *Phytochemistry*, *2,* 395.

Erlenmeyer, H., & Schoenauer, W. (1937). *Helvetica Chimica Acta*, *20,* 1008.

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Gill, P. M. W., Johnson, B. G., Robb, M. A., Cheeseman, J. R., Keith, T., Petersson, G. A., Montgomery, J. A., Raghavachari, K., Al-Laham, M. A., Zakrzewski, V. G., Ortiz, J. V., Foresman, J. B., Cioslowski, J., Stefanov, B. B., Nanayakkara, A., Challacombe, M., Peng, C. Y., Ayala, P. Y., Chen, W., Wong, M. W., Andres, J. L., Replogle, E. S., Gomperts, R., Martin, R. L., Fox, D. J., Binkley, J. S., Defrees, D. J., Baker, J., Stewart, J. P., Head-Gordon, M., Gonzalez, C., & Pople, J. A. (1995). *GAUSSIAN 94 (Revision F.1)*. Pittsburgh PA: Gaussian, Inc.

Hutchinson, J. and Dalziel, J.M. In: Flora of west tropical Africa, Vol. 2, 2nd ed., F.N. Hepper (Ed.). Crown Agents for Oversea Governments and Administrations, London, 1963, p. 125.

Ickes, G. R., Fong, H. H. S., Schiff Jr., P. L., Perdue Jr., R. E., & Farnsworth, N. R. (1973). *Journal of Pharmaceutical Science*, *62,* 1009.

Jensen, S. R. (1983). *Phytochemistry*, *22,* 1761.

Knapp, J. E., & Schiff Jr., P. L. (1971). *Journal of Pharmaceutical Science*, *60,* 1729.

Mills, G. C., Davis, N. J., & Lertratanangkoon, K. (1989). *Nucleosides Nucleotides*, *8,* 415.

Rauhut, G., Alex, A., Chandrasekhar, J., Steinke, T., Sauer, W. Beck, B., Hutter, M., Gedeck, P., & Clark, T. VAMP 6.1; available from Oxford Molecular Ltd, The Medawar Centre, Oxford Science Park, Sandford-on-Thames, Oxford, OX4 4GA, UK. .

Srikrishnan, T., Parthasarathy, R., Alderfer, J. L., Dutta, S. P., & Chheda, G. B. (1988). *Nucleosides Nucleotides*, *7,* 45.

Anon. (1989). *The Merck Index*, (11th ed.). Rahwey, New Jersey: Merck.

Wolinski, K., Hilton, J. F., & Pulay, P. (1990). *Journal of the American Chemical Society*, *112,* 8251.